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UPTAKE OF Zn^{65} AND PRIMARY PRODUCTIVITY IN MARINE BENTHIC ALGAE[1]

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ABSTRACT

The rate of uptake of Zn^{65} from natural sea water and the primary production of 6 species of marine benthic algae were measured in light and dark bottles suspended in a running sea water aquarium under constant light and temperature conditions. Uptake and production of one species (*Chaetomorpha Linum*) were also measured in bottles suspended at different depths in a marine pond in order to achieve a gradient of light intensities. Definite uptake occurred in the light, but no measurable uptake occurred in the dark. The initial rate of uptake in the light was proportional to the gross oxygen production which varied with the species. The apparent equilibrium uptake rates in *Chaetomorpha* at different light intensities were proportional to the net oxygen production. The data indicated that in marine seaweeds zinc is taken up in proportion to the gross oxygen production and accumulated in proportion to the net oxygen production. Consequently Zn^{65} has possibilities as a tool for the measurement of primary productivity.

Zinc occurs in natural sea water as a minor constituent of the total salt content. In a weekly series of determinations over a period of a year at Beaufort, N. C., Chipman *et al.* (1958) found a range of 1.2 to 19.6 μg/L with a yearly average of 10.8 μg/L. The potential biological importance of zinc in the sea may be great since there is evidence that it is an essential micronutrient element for the growth of plants. A lack of available zinc in soils has long been indicated as a factor in certain crop diseases (Thorne 1957) while Arnon (1958) has demonstrated its importance in culturing experiments with terrestrial plants. In a review of algal physiology, Myers (1951) cites evidence which indicates that zinc is a growth requirement for some species of algae.

In light of the potential importance of zinc as a trace metal in the sea the following series of experiments were executed to determine the pattern and rates of uptake of zinc by marine macroscopic benthic algae. Such information is of interest in connection with the basic zinc cycle in the sea and the possible fate of radioactive contaminants. Also, if the rates of uptake of this element are related to the metabolic activities of the algae, it might be possible to adapt zinc uptake determinations to the measurement of metabolism.

The gamma-emitting isotope, Zn^{65}, was utilized as a tracer in these experiments since it provided a sensitive means of measuring uptake at the low concentrations involved in the sea. The benthic marine

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algae served as convenient test materials due to the availability of several different species and the ease with which they could be utilized in a simple experimental set-up. These experiments are a sequel to similar work with P^{32} carried out by Odum *et al.* (1958), and constituted one of the staff-student research projects which were a part of the 1959 Marine Ecology Course at the Marine Biological Laboratory, Woods Hole, Massachusetts. We are indebted to Dr. T. R. Rice for important suggestions concerning interpretation of the data.

METHODS

In all experiments approximately 0.5 g oven-dry weight of fresh, actively growing algae were placed in 250 cc glass-stoppered bottles along with freshly collected sea water from the Woods Hole harbor. The Zn^{65} tracer solution was obtained from the Oak Ridge National Laboratory on July 12, 1957, as "Zn-65-P Processed, High Specific Activity" at which time it had a specific activity of 789 mc/g of zinc. Allowing for about two years radioactive decay, the amount of tracer introduced into each bottle was about 3.7 μc, and the amount of total zinc added about 37 μg.

In the first type of experiment the bottles were suspended in a running sea water aquarium at constant temperature (approximately 22°C) and were allowed to swing in the current in order to achieve a gentle mixing of the contents. Illumination was provided from the sides by 6 fluorescent tubes which produced an intensity of about 350 ft-c. Dark bottles were suspended in the same manner except that they were covered with aluminum foil in order to exclude all light. In the second group of experiments the bottles were suspended at different depths in a marine pond in order to obtain a gradient of light intensities. Uptake was interpreted as the loss of radioactivity from the water after preliminary experiments showed that there was no measurable adsorption by the glass surfaces of the bottles. With the exception of some of the initial experiments, which were run for several hours, the water samples were taken at 0, 0.5, 1.0, 2.0, and 3.0 hr. At each period the bottles were removed from the bath or pond, shaken gently to insure through mixing, and three aliquots of 0.5 cc each were removed with a syringe and the activity counted with a well scintillation counter. Under these conditions the measured uptake could include actual uptake by the plant cells, uptake by the epiphytic bacteria, or adsorption by the surfaces of the algae.

Net productivity, respiration and gross productivity for the three-hour period were determined by measuring the oxygen concentration by the Winkler method at the beginning and the end of the experiment. After each small withdrawal of water for radioactivity determination, the loss was replenished with an equal amount of sea water in order to avoid the formation of an air space in the bottle.

RESULTS

A measurable uptake of Zn^{65} occurred only in those bottles which were exposed to light. The dark bottles showed no significant change in the activity of the water not only in the 3-hr experiments, but also in those which lasted from 6 to 13 hr. In contrast, those bottles exposed to the light in the aquarium or in the pond showed a marked decrease in the activity of the water. Removal of the aluminum foil from the dark bottles and subsequent exposure to the light resulted in an uptake typical of those algae which were illuminated from the start.

The data from a long-term experiment with *Chaetomorpha Linum* in a light bottle are presented in Figure 1 and can be considered to be representative of the type of curves obtained. The rate of change of activity in the water was greatest at the start of the experiment and continued to decrease until it appeared to approach a constant value. Unfortunately, as these values approach the background rate of the counter (300 cpm) it becomes impossible to determine if a constant value was ever reached.

To compare the uptake rates between different experiments objectively, it would be desirable to know what factors determine the shape of the curve and consequently

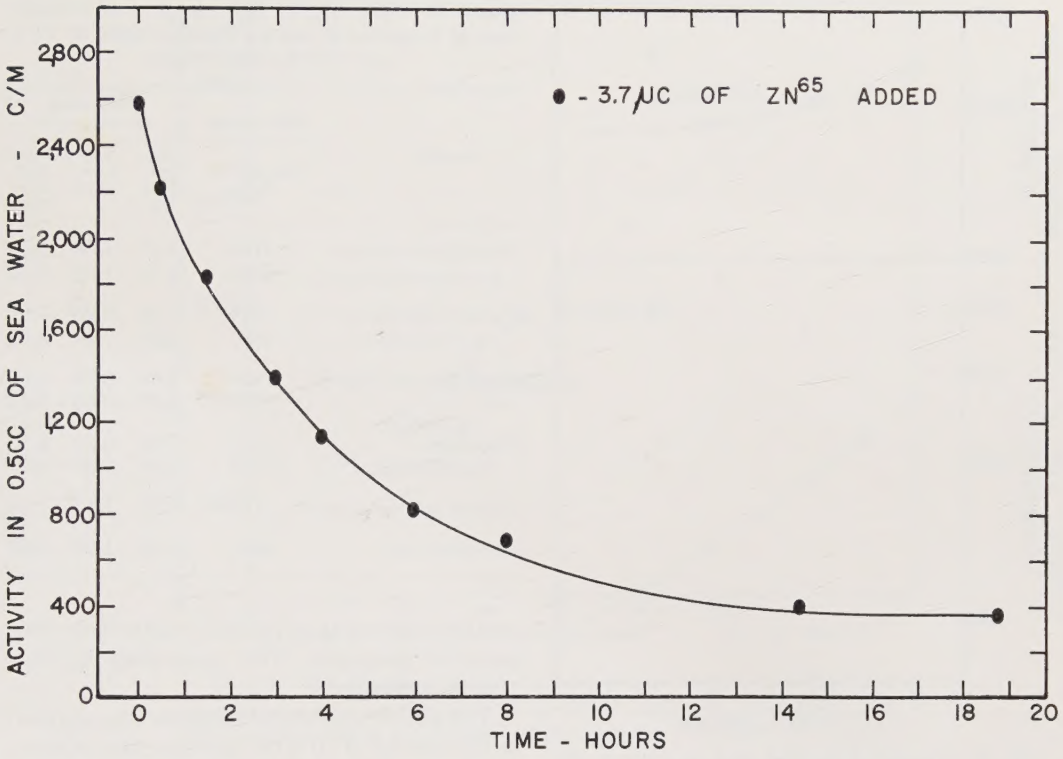

FIG. 1. Typical uptake curve showing loss of Zn^{65} from sea water in a 250 ml "light" bottle containing about 0.5 g (dry wt.) of *Chaetomorpha Linum*.

what parameters are being measured. One possibility would be that zinc is taken up in direct proportion to its concentration in the water. If this were the case, a semi-logarithmic plot of the activities against time would yield a straight line. It was found, however, that in all cases such a plot yielded a curve which was concave (Fig. 2). Another alternative would be that the rate of uptake of zinc was constant during the experimental period, but that a back diffusion of zinc from the algae was reducing the specific activity (defined as the ratio of radioactive to non-radioactive atoms) of the zinc solutions. If a portion of this backflow were Zn^{65} being recycled, this could account for the shape of the curves found. While this theory cannot be conclusively proved, it was found that a backflow of labeled zinc did exist. This was shown by taking pieces of *Ceramium rubrum* which had been labeled with Zn^{65} and placing them in two bottles of fresh sea water. One was placed in the light and the other was covered with aluminum foil. Initial water samples showed some radioactivity in the water at the start of the experiments which was presumably carried over by the water wetting the plants at the time of transfer. During the next 14 hr the activity of the water was reduced in the light bottle while that in the dark bottle showed a constant increase.

In the light of the above information it was assumed that the changing slopes of the uptake curves were due in part to a back flow of zinc from the plants. Since no information was available on the sizes or turnover rates of the zinc pools within the algae, a simple comparison between the curves could not be made. Under the experimental procedure used, however, the initial specific activities of the sea water solutions should have been the same in all of the experimental bottles. The slopes of the uptake curves at time zero should thus provide the most objective values for compar-

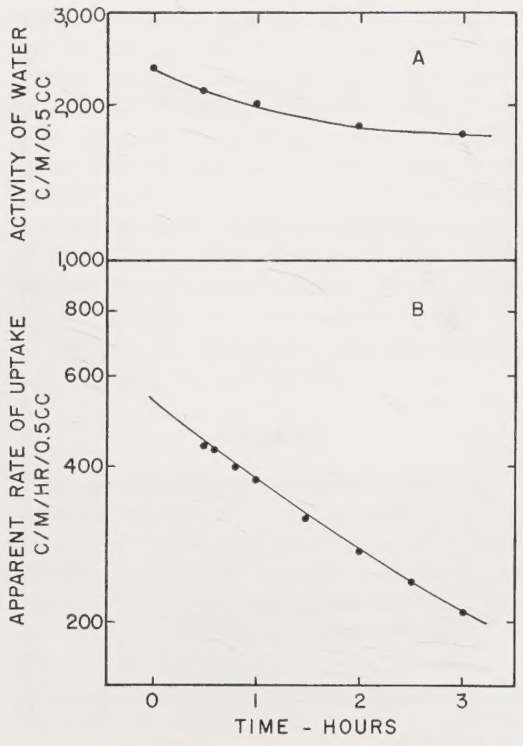

FIG. 2. Graphical method used to estimate the initial rate of uptake of Zn^{65}. Curve A represents the experimental points with a fitted curve. Curve B is a plot of the apparent rates of uptake against the times for which they were computed. The extrapolated rate at time zero (530 c/m/hr/0.5 cc) was considered to be the best estimate of the initial rate of uptake.

ing the uptake curves between different experiments, since the slopes would be directly proportional to the rates of uptake of zinc. Estimates of these initial slopes were made in the following manner. The uptake curves were plotted on semi-logarithmic paper and a line was fitted to the points to form a smooth curve. Lines were then drawn from the activity at time zero to a series of points on the line. The slopes of these lines were then computed as the apparent rates of uptake. These rates were then plotted against the times for which they were calculated, and since as the time approaches zero the rate will approach the initial rate, an extrapolation of a line fitted to these points should yield an estimate of the initial rate. The value for the initial rate was then divided by the weight of algae

TABLE 1. *Summary of Zn^{65} uptake and metabolism of 6 species of marine benthic algae at 22°C and 350 ft-c illumination*

Species	Zn^{65} uptake	Metabolism ml of O_2/g/hr		
	c/m/gm/hr /0.5cc	Net production	Respiration	Gross production
Ceramium rubrum	4746	4.49	1.30	5.79
	5058	6.00	1.30	7.30
Fucus vesiculosis	1641	1.00	0.96	1.96
	1867	1.05	0.96	2.01
Porphyra umbilicalis	2960	3.04	0.89	3.93
	2792	2.63	0.89	3.52
Chordaria flagelliformis	1916	2.22	0.37	2.59
	2322	2.50	0.37	2.87
Chaetomorpha Linum	3197	1.71	1.45	3.16
Laminaria sp.	2565	1.65	1.27	2.92

used to arrive at a relative value for comparative purposes. This procedure is illustrated in Figure 2.

The above method of analysis was applied to the results of 10 3-hr experiments utilizing *Ceramium rubrum, Fucus vesiculosis, Porphyra umbilicalis, Chordaria flagelliformis, Chaetomorpha Linum,* and *Laminaria* sp. The calculated uptake rates and oxygen production values are given in Table 1. The rates of zinc uptake and gross oxygen production for each experiment are plotted in Figure 3. On the basis of the apparent straight line relationship between these two variables a regression line was calculated by the least squares method. A coefficient of correlation was also calculated which gave a value of 0.968. It thus appeared that some type of relationship might exist between the rates of zinc uptake and the gross oxygen production with these taxonomically diverse species.

There is the possibility, however, that these two variables are independent of each other but are dependent on some common factor which varies between species. In order to check this point a single species, *Chaetomorpha Linum,* was on two occasions subjected to a range of light conditions in the field in order to produce a range of metabolic rates in the same species. The data for

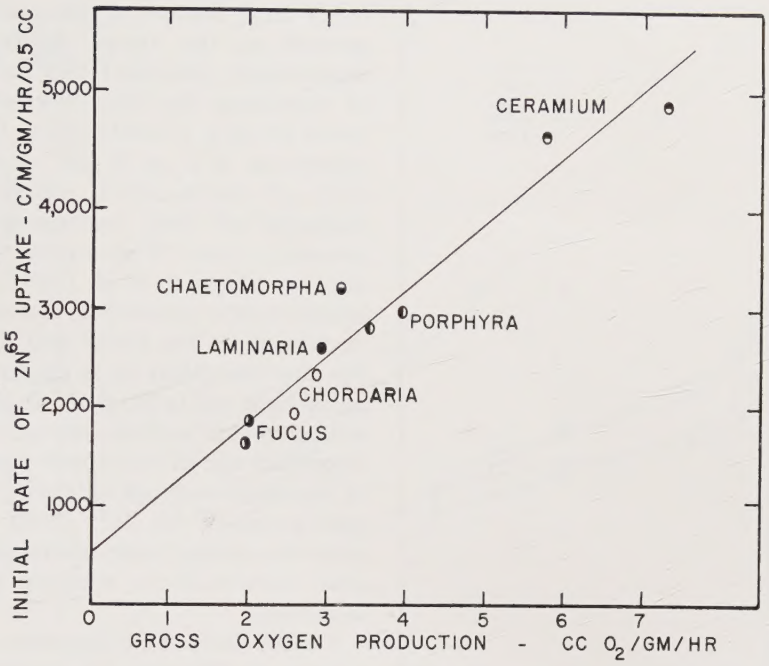

FIG. 3. Relationship between the initial rate of uptake of Zn^{65} and the gross oxygen production of 6 species of marine benthic algae. The least squares line is represented by the equation $Y = 663.8\ X + 513.5$.

the August 29, 1959, experiment are shown in Figure 4. It can be seen that these curves are characterized by a very rapid drop within the first hour, and, with the exception of the 3 m bottle, an apparent linear decrease in activity during the next two hours. Since there were no samples taken at the end of the first half hour, the initial shape of the curve during the first hour is uncertain so that one cannot apply the same methods of analysis which were used in the laboratory experiments. The form of these curves suggested that under the light intensities found in the field the rate of zinc uptake was of such a magnitude as to bring the specific activity of the exchangeable zinc in the algae into equilibrium with that in the sea water within the first hour. The straight line decrease could thus represent a net uptake of zinc in the algae. Going on this assumption, the data were analyzed in the following manner. A straight line was fitted to the last three points and extrapolated to the zero time axis. The slope of the line was then determined and expressed as a percentage of the extrapolated value for comparative purposes. Percentage uptake for both pond experiments are given in Table 2 together with the gross and net oxygen values. It was found that if the uptake rates were plotted against the gross oxygen production in each experiment, they formed a straight line

TABLE 2. *Summary of zinc uptake and metabolism of* Chaetomorpha Linum *suspended at different depths in a marine pond*

Date	Depth m	Zinc uptake %/g/hr[1]	Metabolism ml of O_2/g/hr		
			Net production	Respiration	Gross production
VIII-21-59	0	29.5	1.54	0.95	2.49
	0.5	41.2	2.07	0.95	3.02
	1.0	27.0	1.48	0.95	2.43
	1.5	21.9	1.23	0.95	2.18
VIII-27-59	0.5	26.3	1.34	1.32	2.66
	1.0	22.6	1.07	1.32	2.39
	1.5	29.0	1.20	1.32	2.52
	2.0	15.5	0.84	1.32	2.16
	3.0	16.9	0.79	1.32	2.11

[1] See text for method of calculation.

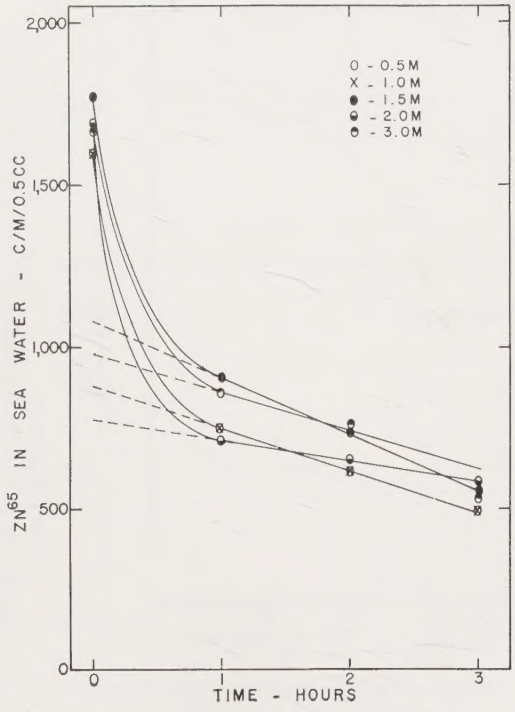

FIG. 4. Uptake of Zn^{65} by *Chaetomorpha Linum* suspended at the indicated depths in a marine pond on August 27, 1959. Values not corrected for the weights of algae used.

which intercepted the oxygen axis at or near to the experimental value for respiration. Thus the apparent net uptake of zinc appears to be related to the net oxygen production. A plot of these values for both experiments is given in Figure 5 along with a calculated regression line. In this case there was a coefficient of correlation of 0.955.

DISCUSSION

The loss of Zn^{65} from the sea water could be due either to physical adsorption on the surfaces of the algae and their associated epiphytes or to actual uptake into the algal cells. It is probable that the latter was what was measured in these experiments due to the facts that no uptake could be detected in the dark bottle controls and that rates of uptake were correlated with oxygen production indicating that a metabolic process was involved. The extent to which adsorption took place was obscured by the relatively large amount of non-radioactive zinc present in the tracer solution. In the experiments described this had the effect of increasing the zinc concentration from about 10 μg/L to about 150 μg/L. Thus the adsorption of 5 μg of zinc would result in only a 3% loss in activity which could not be distinguished from normal sampling and counting errors. When carrier free Zn^{65} was used by Chipman *et al.* (1958) to measure uptake in the planktonic diatom, *Nitzschia closterium*, it was found that about 80% of the zinc was taken up in the first hour both in the light and in the dark. In their case it is indicated that surface adsorption played an important role in zinc uptake since by means of repeated washings of labeled cells a large percentage of the Zn^{65} could be removed with the amount removed increasing as the zinc content of the washing medium was increased.

On the basis of the apparent straight line relationship between zinc uptake and oxygen production, it is suggested that zinc is taken up in direct proportion to the photosynthetic rate, and that it accumulates in the algae as a function of their growth or net production. Further research is thus indicated to test this hypothesis and if found true to establish a zinc to oxygen ratio in absolute terms. If such a relationship can be shown to be valid for several species over a wide range of conditions, Zn^{65} might provide still another tool for the estimation of primary production. This radionuclide would be of particular value under certain circumstances since its gamma radiations eliminate the self-adsorption problems found with other tracers commonly employed in productivity studies.

REFERENCES

ARNON, D. I. 1938. Microelements in culture solution experiments with higher plants. Amer. Jour. Bot., **25**:322–325.

CHIPMAN, W. A., T. R. RICE, AND T. J. PRICE. 1958. Uptake and accumulation of radioactive zinc by marine phytoplankton, fish, and shellfish. U. S. Fish and Wildlife Service Fish. Bull., 58(135):279–292.

MYERS, J. 1951. Physiology of the algae. Ann. Rev. Microbiol., **5**:157–180.

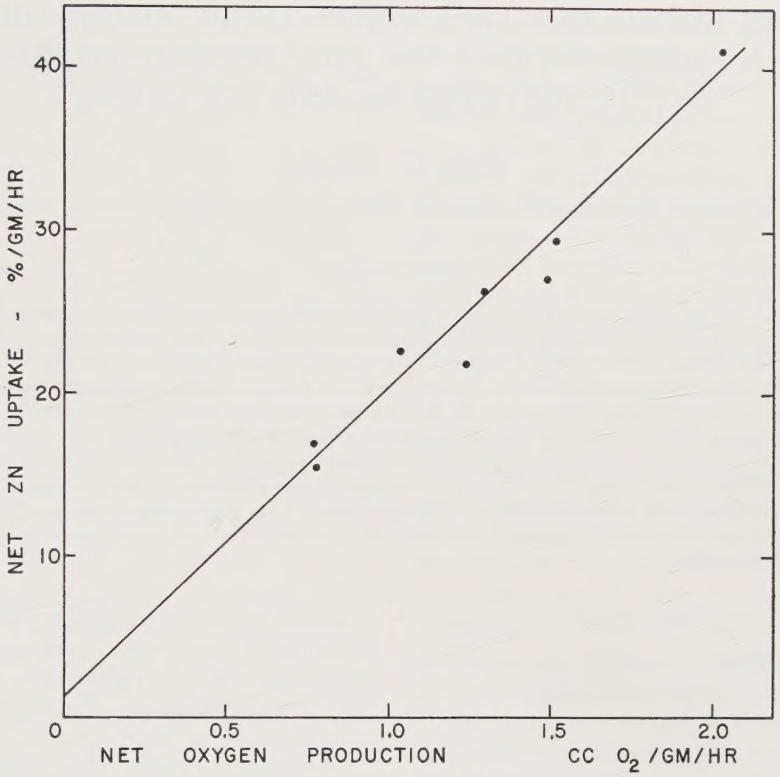

FIG. 5. "Equilibrium" uptake rates of *Chaetomorpha Linum* plotted against the net oxygen production. The least squares line is represented by $Y = 18.8\ X + 1.4$.

ODUM, E. P., E. J. KUENZLER, AND SISTER MARION XAVIER BLUNT. 1958. Uptake of P^{32} and primary productivity in marine benthic algae. Limnol. Oceanogr., **3**:340–345.

THORNE, W. 1957. Zinc deficiency and its control. *In* Advances in Agronomy, Norman, A. G. (*Ed.*). Vol. IX pp. 31–65.

THE LIMNOLOGY OF CANYON FERRY RESERVOIR: III. SOME OBSERVATIONS ON THE DENSITY DEPENDENCE OF PHOTOSYNTHESIS AND ITS CAUSE[1]

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ABSTRACT

A series of 24-hr determinations of net photosynthesis confirmed the conclusions derived from previous 6-hr experiments that the relationship between photosynthesis and standing crop is not linear. The optimal rate of net photosynthesis per unit chlorophyll varied inversely with chlorophyll concentration. As a result net euphotic zone photosynthesis was maximal at intermediate values of chlorophyll concentration and was less at chlorophyll concentrations above or below the intermediate range. From previously derived relationships between chlorophyll, gross photosynthesis, and respiration, values of net euphotic zone photosynthesis were computed for each chlorophyll concentration. The computed values agreed well with the observed values. The decline of CO_2 concentration as standing crop increased was suggested as the most probable cause of the observed photosynthesis standing crop relationship.

INTRODUCTION

The estimation of net primary production is of great importance, since only the amount of carbon that is fixed in excess of the respiratory requirements of phytoplankton is available to higher trophic levels. In a previous paper (Wright 1959) an attempt was made to measure net primary production per unit chlorophyll. This was done by correcting observed rates of gross photosynthesis for the amount of respiration that could be expected on the basis of chlorophyll content. Respiratory rates were obtained by measuring the rate of respiration per unit chlorophyll of phytoplankton concentrates. It was observed that the rate of gross photosynthesis per unit chlorophyll decreased as the chlorophyll concentration became greater. As a result, when net production per unit of lake surface was compared with the size of the standing crop of chlorophyll, maximum net production occurred within a certain range of chlorophyll standing crops and became less as chlorophyll decreased or increased.

However these conclusions could be criticized since they were based on 6-hr exposures and on phytoplankton populations that were a mixture of samples taken at various depths from the euphotic zone. Consequently during May, 1959, 6 determinations were made of net photosynthesis over 24-hr periods. The results were compared with values that could be expected from the 6-hr series.

METHODS

In 250 ml steam-sterilized, glass-stoppered pyrex bottles, the samples were suspended at the depths from which they were taken. Sampling was done at 5-ft intervals from the surface to 30 ft. The bottles were suspended between 1300 and 1500 hr and were taken up 24 hr later. The chlorophyll content of each sample was determined and net photosynthesis was measured by computing the difference between initial and final oxygen content. On each sampling date, light penetration data were obtained with a submarine photometer. The sampling dates covered the range between the maximum and minimum spring diatom populations.

RESULTS

The observed daily rates of optimal net photosynthesis were divided by 24, giving

[1] Field work for this study was supported by a National Science Foundation grant. The data were processed and the manuscript prepared during the tenure of a National Science Foundation Senior Postdoctoral Fellowship at Osborn Zoological Laboratory, Yale University, New Haven, Connecticut.

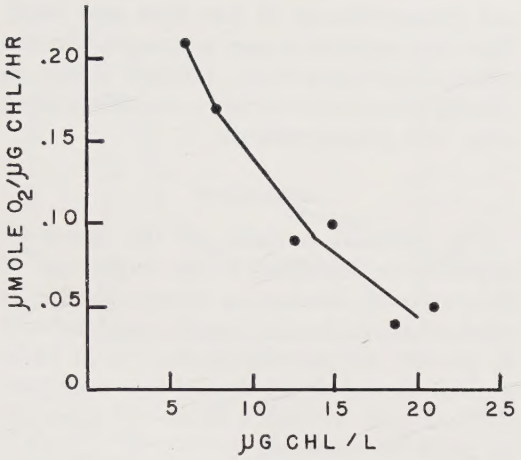

FIG. 1. Average hourly rates of optimal net photosynthesis compared with chlorophyll concentration.

average hourly rate over the 24-hr period (Table 1). The results are also presented in graphical form in Figure 1. It will be noted that chlorophyll-based rates of optimal net photosynthesis consistently declined with increasing size of chlorophyll standing crop. The results are also compared with rates that could be expected from the results given by Wright (1959). These computed rates were determined in the following manner. From Table 1 (Wright 1959) rates of hourly optimal gross photosynthesis corresponding to the chlorophyll concentrations listed in Table 1 of this paper were selected. A 12-hr photosynthesis period was assumed and the rates were multiplied by 12 to give daily optimal gross photosynthesis per unit chlorophyll. In order to get the daily rate of respiration the average rate of respiration (0.033 μmoles/μg chl/hr) was multiplied by 24. The difference between daily rate of optimal gross photosynthesis and respiration per unit chlorophyll was divided by 24; this yielded the expected hourly rate of optimal net photosynthesis over a 24-hr period. The average of the observed rates was 0.11 μmole O_2/μg chl/hr which compares very favorably with the computed rate of 0.13 μmoles O_2/μg chl/hr. However, reference to Table 1 reveals a consistent deviation of observed daily rates from computed rates when standing crops of chlorophyll were large. The discrepancy may have been due to a higher rate of community respiration, a higher rate of phytoplankton respiration, or to an exaggeration of the effect of crop size on photosynthesis when the plankton was confined to the bottle for longer periods of time. Measurements of photosynthesis using C^{14} might be useful in resolving this kind of situation since the method apparently measures net photosynthesis.

Observed values of net euphotic zone photosynthesis are given in Table 1 and graphically presented in Figure 2. These are compared with values computed following the conclusions reached in Wright (1959) and according to equation (1).

$$\Sigma P_{net} = \frac{4.6}{k} (F \times T \times chl \times R_{opt} - 0.033 \times 24 \times chl) \quad (1)$$

Calculated from the 6-hr determinations made during May of 1957 and 1958, the value of F was the average ratio of euphotic zone photosynthesis to optimal photosyn-

TABLE 1. *Observed and computed rates of optimal and euphotic zone net photosynthesis*

Date 1959	Extinction Coefficient	Average euphotic zone chlorophyll content (μg/L)	Optimal rate of gross photosynthesis per unit chlorophyll (μmole O_2/μg chl/hr)	Average hourly rate of optimal net photosynthesis per unit chlorophyll (μmole O_2/μg chl/hr)		Average daily rate of euphotic zone net photosynthesis (mmole O_2/m^2/day)	
				Computed	Observed	Computed	Observed
May 4	0.56	18.4	0.26	0.10	0.04	89	43
May 7	0.54	21.0	0.22	0.08	0.05	67	99
May 14	0.48	14.8	0.30	0.12	0.10	113	123
May 21	0.54	12.5	0.32	0.13	0.09	96	104
May 25	0.60	5.8	0.43	0.20	0.21	75	86
May 28	0.51	7.6	0.38	0.16	0.17	88	104
Average	0.54	13.4	0.32	0.13	0.11	87	93

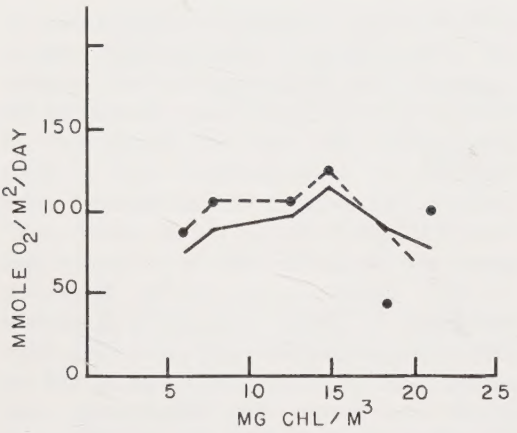

FIG. 2. Daily euphotic zone photosynthesis compared with chlorophyll concentration. The dotted and dashed line represent measured values. The solid line represents computed values.

thesis on a unit volume basis. This value (0.44) compared very well with the average ratio of 0.47 which was calculated from the 24-hr series. A 12-hr photosynthetic period (T) was used in the calculations. Values for chlorophyll standing crop were obtained by averaging the chlorophyll content of the various depths sampled. Optimal rates of gross photosynthesis (R_{opt}) were selected to correspond with the average chlorophyll concentration. The expression ($0.033 \times 24 \times$ chl) is the daily amount of respiration per unit volume in the euphotic zone.

The average computed value of 87 mmole $O_2/m^2/day$ was very close to the observed value of 93. Reference to Figure 2 shows that observed values of net euphotic zone photosynthesis closely follow the computed values if the curve is drawn through the average of the observed values associated with the two largest crops of chlorophyll. The maximum rate of observed net photosynthesis occurred at approximately 15 mg chl/m^3 which coincides with the size of standing crop that could be expected to deliver maximum net photosynthesis. It is also noteworthy that the standing crop can fluctuate within wide limits without greatly affecting the rate of net photosynthesis per unit surface area. For example a nearly threefold change in standing crop from 15 to 5.8 mg chl/m^3 resulted in a reduction of net photosynthesis of less than one third. This also explains in part why such low correlation coefficients are obtained when an attempt is made to correlate size of standing crop with photosynthesis.

DISCUSSION

The original purpose of the series of experiments described in this paper was to determine if conclusions drawn from 6-hr photosynthesis measurements could be used to predict net photosynthesis over a 24-hr period. It was demonstrated that such predictions have as high a degree of accuracy as could be expected.

It is apparent that one cannot assume that the rate of optimal photosynthesis per unit standing crop is constant; instead it is density dependent. Allowance for this fact reduces some of the scatter between observed and predicted values of photosynthesis. The rate of optimal photosynthesis is affected by other factors, such as the interrelationship between temperature, light and photosynthesis which is correlated with seasonal succession of phytoplankton (Verduin 1956, Ichimura and Aruga 1958, Rohde *et al.* 1958, and Wright 1959). The existence of a nutrient effect has also been demonstrated by Ichimura and Aruga (1958). Because of this complicated array of factors it is not possible, on the basis of present knowledge, to predict accurately the rate of photosynthesis for a particular date and one must be content with averages (Verduin 1959).

It also seems logical that the density dependence of photosynthesis which has also been observed by McQuate (1956), Rohde *et al.* (1958) and Verduin (1959) must be understood before attempts are made to untangle this complex situation. The question naturally arises as to why the specific photosynthesis rate is inversely proportional to the size of the standing crop whether the latter is expressed as chlorophyll concentration or cell volume.

Three alternative explanations present themselves: 1) as determined spectrophotometrically, the apparent content of chlorophyll consists of an increasing amount of degradation products whose absorbance

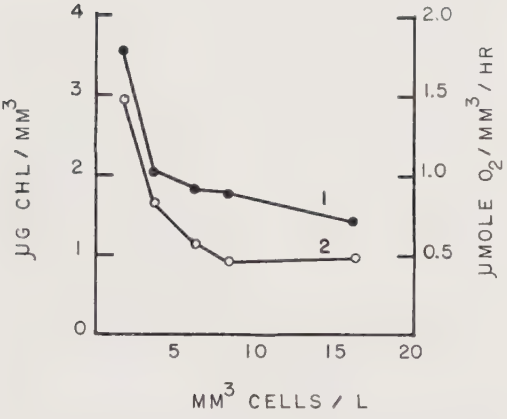

FIG. 3. The ratio of chlorophyll content to cell volume (Curve 1) and the ratio of optimal gross photosynthesis (Curve 2) to cell volume.

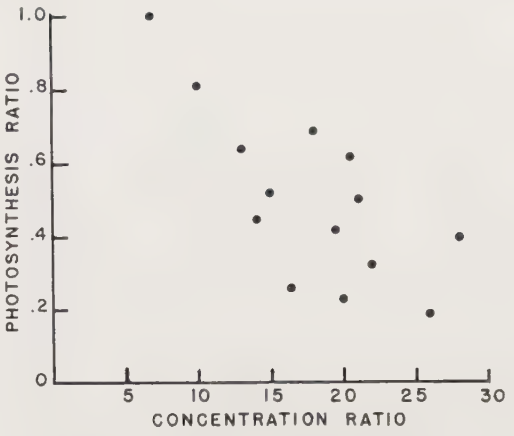

FIG. 4. The ratios of gross photosynthesis of concentrated phytoplankton to unconcentrated phyto plankton compared with the ratios of concentration.

spectrum is so similar to chlorophyll that they cannot be distinguished spectrophotometrically (Brown, *Personal Communication*); 2) photosynthetic inhibitors similar to chlorellin (Pratt 1943) accumulate as the population increases; 3) the nutrient content of the water decreases to such a low level that the rate of supply of substances necessary for the photosynthetic reaction limits the rate of photosynthesis without a proportional decrease in chlorophyll.

Figure 3 gives a comparison between chlorophyll content and optimal photosynthesis on a cell volume basis. The data were taken from Table 1 of Wright (1959). It is apparent that as cell volume increases the chlorophyll content per unit cell volume drops rapidly at first and then tends to level off. The curve for photosynthesis per unit cell volume behaves similarly. Hence part of the explanation for the inverse relationship between photosynthesis per unit cell volume and size of crop lies in the fact that the chlorophyll content varies inversely. This relationship strongly suggests that chlorophyll synthesis becomes increasingly limited by a nutrient factor (probably nitrogen) as the crop increases.

It will be noted (Figure 3) that in the range between 4 and 8 mm^3 cells/L the rate of photosynthesis decreases more rapidly than the chlorophyll content. This could be due to an overestimate of chlorophyll because of the presence of degradation products. On the other hand, the asymptotic leveling off of the photosynthesis curve strongly suggests the presence of another limiting factor besides chlorophyll. The curve should have continued downward beyond 8 mm^3 cells/L if chlorophyll degradation products were involved.

During 1957 and 1958, measurements were made of gross and net photosynthesis of phytoplankton that had been concentrated by towing a #20 Wisconsin net through the water. The bottles containing the concentrates were suspended at depths where optimal photosynthesis of natural populations could be expected. In Figure 4 the ratios of photosynthesis of the 1958 concentrates to that of the natural populations are plotted against the ratios of concentration. Increasing the concentration of the phytoplankton resulted in a lowering of the photosynthetic rate and thus duplicated the inverse relationship between standing crop and photosynthesis previously described. This evidence clearly implies that some external factor becomes limiting to photosynthesis as the crop becomes larger. The probable nature of the limiting factor becomes evident from an examination of Figure 5 in which average chlorophyll concentration, gross photosynthesis, and net photosynthesis are compared with pH val-

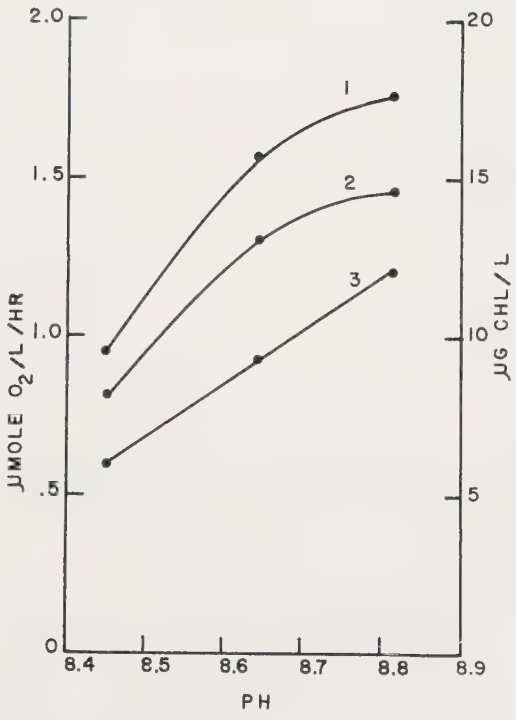

FIG. 5. Gross euphotic zone photosynthesis (Curve 1), net euphotic zone photosynthesis (Curve 2), and chlorophyll concentration compared with pH.

ues, which are averages of various ranges and represent the average pH at the start of a 6-hr black-and-clear bottle experiment. It will be noted that there was a linear relationship between pH and chlorophyll concentration (Curve 3). It is apparent that as crops became larger the pH increased as a result of removal of CO_2 by the photosynthetic reaction. Curve 1 relates hourly gross photosynthesis to pH and rapidly decreases in slope at the higher pH values. Curve 2 portrays the relationship between hourly net photosynthesis and pH. The rate of decrease in slope is much greater and it can be seen that the curve is asymptotic at the highest pH. The conclusion seems inescapable that the density dependence of photosynthesis is due to a decrease in available CO_2 as the crop becomes larger. It seems to have been taken for granted, by all workers (including the author) in this field, that there are always sufficient bicarbonate ions in alkaline waters to supply adequate CO_2 for the photosynthetic reaction. Yet Burr (1941) demonstrated the limiting effect of CO_2 tension on photosynthesis of algae and other aquatic plants and stated the following: "To recapitulate, solutes, temperature, light, and carbon dioxide tension operate simultaneously on the photosynthetic mechanism, and in any attempt to describe the environment of aquatics all four should be recorded with the greatest possible precision."

It seems likely that the morphometry of a lake will strongly affect the degree with which CO_2 tension limits photosynthesis. Verduin (1956) has shown in western Lake Erie that the nocturnal pH decrease is equal to the diurnal pH increase, indicating that community respiration replaces the CO_2 absorbed in photosynthesis. However western Lake Erie is shallow and unstratified, hence CO_2 released by the entire community is readily transferred to the euphotic zone by turbulence. The situation is entirely different in a deep stratified lake such as Canyon Ferry located in a steep-walled canyon. In this case the return of CO_2 to the euphotic zone will be impeded during the summer period of thermal stratification. It may well be that an upper limit to net photosynthesis is set by the rate at which CO_2 becomes available through epilimnetic respiration, turbulent transfer, advection, and diffusion from air. The magnitude of the upper limit in different lakes should vary according to the morphometry of the lake and the content of the other nutrients such as phosphate and nitrate.

The situation in Canyon Ferry may be analyzed further by reference to Figure 6. In this figure size of standing crop is compared with potential optimal gross photosynthesis (Curve 1) and actual optimal gross photosynthesis (Curve 2). The first curve was constructed by determining the average amount of chlorophyll that could be expected to occur for averages of various ranges of cell volumes. The amount of chlorophyll determined in this manner was then multiplied by the rate of optimal gross photosynthesis per unit chlorophyll that occurred in crops of the smallest average size. Thus the curve expresses the limiting

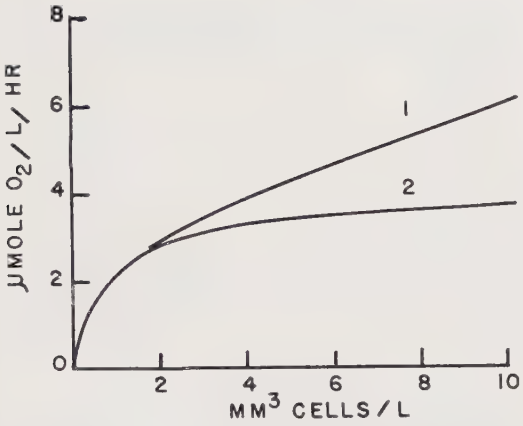

FIG. 6. Curve 1 represents the rate of optimal gross photosynthesis that could be expected to occur on the basis of the chlorophyll content of crops of varying cell volume. Curve 2 represents actual observed values of optimal gross photosynthesis compared with cell volume.

effect on photosynthesis of nutrients that are necessary for the synthesis of chlorophyll. If nothing else limited the photosynthetic reaction, the rate of photosynthesis plotted against cell volume should follow Curve 1. This does not prove to be the case as examination of Curve 2 will show. The greater rate of decrease in slope of this curve is apparently due to the limiting effect of CO_2 tension on the rate of photosynthesis. The difference between the two curves may be considered a measure of the magnitude of the limitation imposed by CO_2.

It seems obvious that various shapes of curves would be obtained if an examination of different situations were made. This would depend upon the relative relationship between the supply of nutrients required for chlorophyll synthesis and the CO_2 requirement for photosynthesis. In any case, however, it seems likely that when the phytoplankton has utilized fully the nutrient potential of an environment for chlorophyll synthesis, the rate of photosynthesis will become limited by CO_2.

REFERENCES

BURR, GEORGE O. 1941. Photosynthesis of algae and other aquatic plants. *In*: A Symposium on Hydrobiology. Univ. Wisc. Press, Madison. 405 pp.

ICHIMURA, SHUN-EI, AND YUSHO ARUGA. 1958. Some characteristics of photosynthesis of fresh water phytoplankton. The Botanical Magazine, Tokyo, **71**: 841–842.

MCQUATE, A. G. 1956. Photosynthesis and respiration of the phytoplankton in Sandusky Bay. Ecology, **37**: 834–839.

ROHDE, W., R. A. WOLLENWEIDER, AND A. NAWERK. 1958. The primary production and standing crop of phytoplankton. *In*: Perspectives in marine biology, A. A. Buzzati-Traverso (*Ed.*). Univ. Calif. Press, Berkeley. 621 pp.

VERDUIN, J. 1956. Energy fixation and utilization by natural communities in western Lake Erie. Ecology, **37**: 40–50.

———. 1959. Photosynthesis by aquatic communities in northwestern Ohio. Ecology, **40**: 377–383.

WRIGHT, J. C. 1959. Limnology of Canyon Ferry Reservoir. II. Phytoplankton standing crop and primary production. Limnol. Oceanogr., **4**: 235–245.

SALT REQUIREMENTS OF MARINE PHYCOMYCETES[1]

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ABSTRACT

All 49 isolates of nonfilamentous phycomycetes of marine origin were found to require NaCl for optimum growth. *Thraustochytrium globosum* gave parallel responses, which were not qualitatively affected by temperature, to salinity and to NaCl concentration. The NaCl optimum was unchanged after 5 serial cultures, using large inocula, in low (1%) and high (4%) NaCl.

Microorganisms isolated from marine habitats are frequently characterized by salinity optima which approximate to the conditions of the natural habitat (ZoBell 1946, Provasoli 1958). Among the bacteria this phenomenon appears to be primarily the result of an NaCl requirement (MacLeod and Onofrey 1956, 1957), which may be altered during cultivation in the laboratory by mutation (Pratt and Waddell 1959) or by factors not altogether clear (ZoBell and Michener 1938). Higher marine microorganisms may be more fastidious, requiring a nearly complete synthetic sea water (Provasoli 1958). The higher fungi, however, may be active in the ocean by virtue of a broad tolerance for salt rather than as a specialized ecological group specifically adapted to life in the ocean. Many species well known from other than marine habitats tolerate salt concentrations higher than those of sea water (Siepmann 1959). Of the higher fungi, only *Halophiobolus opacus* has been reported as requiring sea water (Gustafson and Fries 1956), though the rate of growth of other fungi may be affected by salinity (Barghoorn and Lindner 1944, Ritchie 1957, 1959). The effect of salinity on growth is in some cases temperature dependent (Ritchie 1957, 1959). We have investigated the NaCl requirements of a variety of nonfilamentous phycomycetes isolated from sea water, attempting to determine whether or not they constitute a specialized ecological group. We have also studied the relationships of salt optimum to changes in temperature and to selection by cultivation at nonoptimal NaCl concentrations.

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METHODS

The 49 isolates used in this study were obtained by plating water samples from Woods Hole, Mass., and Long Island Sound according to previously published methods (Vishniac 1956). They include all the isolates so obtained which have been capable of growth on the synthetic medium of Table 1. Of these isolates 25 have been found to grow well only with the addition of $NaHCO_3$, 0.01% (beyond that naturally formed in the medium), bringing the pH of the medium to approximately 7.8. Adjustment of the medium to this pH prior to autoclaving, or after autoclaving with bases other than $NaHCO_3$, was unsatisfactory. Some of them have been carried in pure cultures since 1954; others were isolated during the succeeding years, some as late as December of 1959. While most of these isolates have not been identified, our observations indicate that they are members of the Ectrogellaceae and Thraustochytriaceae. More detailed study was made of *Thraustochytrium globosum* (isolate L-5) which was isolated in April, 1955, from water from Lighthouse Point, New Haven, Conn.

The NaCl requirements of these isolates were determined using the medium of Table 1, modified in NaCl content, distributed in 5 ml portions in 16 × 125 mm screw-capped pyrex tubes. Such tubes were inoculated

TABLE 1. *Stock medium for marine phycomycetes*

Salts (5× solution yielding final concentrations of)	
NaCl	2.5 %
$MgSO_4.7\ H_2O$	0.5 %
KCl	0.1 %
KH_2PO_4	0.01%
$CaCO_3$	0.02%
H_2SO_4	to dissolve (*ca* 0.05%)
$(NH_4)_2SO_4$	0.02%
NaOH to pH *ca*	6.0
to which is added	
Trace metals M-5 (100× solution yielding final concentration of)	
Na_2EDTA	5.0 mg/100 ml
Fe^{++} (as $FeSO_4.7H_2O$)	0.05 mg/100 ml
Zn^{++} (as $ZnSO_4.7H_2O$)	0.02 mg/100 ml
Mn^{++} (as $MnSO_4.H_2O$)	0.01 mg/100 ml
Co^{++} (as $CoSO_4.7H_2O$)	2.0 μg/100 ml
Cu^{++} (as $CuSO_4.5H_2O$)	0.2 μg/100 ml
B^{+3} (as H_3BO_3)	2.0 μg/100 ml
Mo^{+6} (as $Na_2MoO_4.2H_2O$)	2.0 μg/100 ml
and	
NaH.glutamate	0.2%
Glycerol	0.2%
Agar	0.1%
Thiamine.HCl	1 μg/100 ml
Cyanocobalamin	0.1 μg/100 ml
pH finally adjusted to 7.5 with NaOH	
Glucose (filter sterilized and added to sterile medium)	0.2%
$NaHCO_3$ (autoclaved and added to sterile medium as required)	0.01%

with one drop to 0.5 ml of a stock culture and incubated at 15°C for more than two weeks during which time the tubes were shaken frequently. Growth was followed by visual inspection. Since growth in tubes is limited to the upper centimeter of the medium, presumably more because of access to air than because of the experimental variable, this system is considered somewhat crude. A more sensitive system is provided by using 10 ml of medium in 25 ml pyrex Erlenmeyer flasks, which provided a layer of medium approximately 1 cm deep. Such flasks, capped with beakers and sealed between two pyrex baking dishes with Scotch tape, were used for more precise determinations of the responses of isolates B-1, WH361m, and L-5. The salinity and NaCl optima of L-5 were determined at both 15° and 25°C. Both temperatures allow excellent growth of this fungus. Salinity optima were determined with 2 × concentrated Long Island Sound sea water, salinity *ca* 28‰. The boiled sea water was filtered and enriched with trace metals (M-5 of Table 1), KH_2PO_4 0.005%, $(NH_4)_2SO_4$ 0.02%, NaH.glutamate 0.2%, glycerol 0.1%, agar 0.1%, thiamine HCl 40μg/100 ml, and B_{12} 0.1 μg/100 ml. This enriched sea water was diluted to produce final concentrations of 10 to 180% sea water with a solution of the enrichments only in distilled water at pH 7.5. Cultures of L-5 were allowed to grow for 7 days at 25°C and 10 days at 15°C before reading in the Klett-Summerson colorimeter with No. 42 filter.

RESULTS

When inoculated into media containing 1%, 2.5%, and 5% NaCl, 27 of the 49 isolates made no growth with 1% NaCl and 4 others grew less well with 1% than with 2.5% NaCl. With 5% NaCl, 11 failed to grow, while 7 grew only poorly. The ability of the NaCl requiring isolates of this experiment to grow well with 5% NaCl was correlated with their need for $NaHCO_3$ for optimal growth: of 14 isolates not requiring added $NaHCO_3$ only one grew well with 5% NaCl, while all of the 10 isolates requiring added $NaHCO_3$ grew well at 5% NaCl. The isolates which did not display an NaCl requirement were all capable of growth at 5% NaCl. These isolates were further inoculated into media containing 0.5%, 2.5%, and 6% NaCl. With 0.5% NaCl only 2 (B-1 and WH361m) continued to grow as well as with 2.5% NaCl, although 14 were able to make significant growth. With the exception of one of these isolates, growth was slight or nil at 6% NaCl. These data are summarized in Table 2. When isolates B-1 and WH361m were retested in flasks, using very small inocula (0.05%), both showed clear optima at 2.0% NaCl (Fig. 1). It may therefore be concluded that optimum growth requirements of our entire collection are NaCl concentrations characteristic of sea water rather than of either fresh water or brine pools.

The effect of NaCl concentration and of salinity on the growth of L-5 is shown in Figure 2. It is evident that there is an optimum for growth at 2.5% NaCl, and that

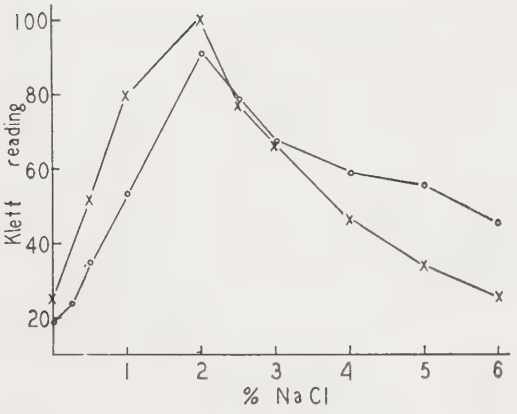

FIG. 1. The response of isolates B-1 (o) and WH361m (x) to NaCl concentration. Culture WH361m was incubated for 4 days and culture B-1 for 5 days at 25°C.

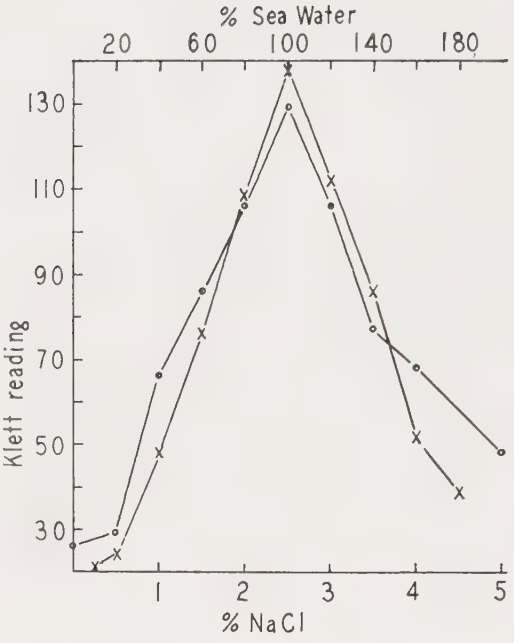

FIG. 2. The effects of NaCl concentration (o) and salinity (x) on the growth of isolate L-5. It was incubated for 7 days at 25°C.

there is good agreement between the effects of media based on sea water and synthetic media. The optimum was not affected by the temperature. These cultures were then used as the starting material for determining the readiness with which such optima may be changed. From the cultures grown at 15°C, 1 ml each from flasks containing 1% and 4% salt were transferred to 5 ml of medium of the same salt content in screw-capped tubes. After a week of further growth 0.1 ml (1×10^5 cells by haemocytometer count) from these tubes was used to inoculate flasks of similar salt content. After 9 days of growth 0.5 ml from the flasks were again subcultured into tubes. Twelve days later these tubes were again subcultured using 0.5 ml/5 ml medium. These tubes were allowed to grow for 25 days, diluted 1:4 with fresh medium, and one drop used as inoculum for redetermining the NaCl optimum. The inoculum was therefore derived from 5 serial cultures in 1% or 4% NaCl, each transfer involving 10^5 to 10^6 cells and growth to 10^6 to 10^7 cells/ml. The response given was substantially unchanged from that of Figure 1. We would presume therefore that mutants with respect to NaCl optimum, if they occur, are rare in this fungus.

These nonfilamentous phycomycetes appear to be a distinct ecological group, physiologically specialized for growth in sea water. The considerable range of NaCl concentrations tolerated and the slightly low salt optimum seen in B-1 and WH361m might be expected in littoral organisms. It

TABLE 2. *Salt requirements of marine phycomycetes*

Origin	Date	No. isolates	No. growing well at 0.5% NaCl	1.0% NaCl	2.5% NaCl	5.0% NaCl	6.0% NaCl
(Dr. S. W. Watson)	1951	1	0	1	1	1	0
Long Island Sound Conn. Shore	Jan. '54	2	1	1	2	1	1
	Apr. '55	2	0	2	2	2	0
	Jul. '58	8	0	4	8	4	0
	Dec. '59	14	0	6	14	9	0
Woods Hole, Mass.	Jun. '56	22	1	8	22	14	0
Total		49	2	22	49	31	1

is surprising that all of this collection should show this response to NaCl, however, since several floristic and ecological studies have referred specimens found in brackish to marine waters to fresh water nonfilamentous species (Kobayashi and Ookubo 1953, 1954; Höhnk 1956). One might infer that the marine forms of these species, if really physiologically distinguishable from their fresh water counterparts, are closely related physiological varieties, of no long evolutionary history. Our failure to select mutants with respect to NaCl optimum argues against such ready interconvertibility as has been found in certain bacteria and consequently against so close a relationship with fresh water forms.

REFERENCES

Barghoorn, E. S., and D. H. Linder. 1944. Marine fungi: Their taxonomy and biology. Farlowia, **1**: 395–467.

Gustafson, U., and N. Fries. 1956. Nutritional requirements of some marine fungi. Physiol. Plant., **9**: 462–465.

Höhnk, W. 1956. Studien zur Brack-und Seewassermykologie VI. Veröff. Inst. Meeresforsch. Bremerhaven, **4**: 195–213.

Kobayaishi, Y., and M. Ookubo. 1953. Studies on the marine phycomycetes. Bull. Nat. Sci. Museum (Tokyo). 1954. **33**: 53–65.

———. 1954. Studies on the marine phycomycetes II. Bull. Nat. Sci. Museum (Tokyo), N.S. **1**: 62–71.

MacLeod, R. A., and E. Onofrey. 1956. Nutrition and metabolism of marine bacteria II. J. Bact., **71**: 661–667.

———. 1957. Nutrition and metabolism of marine bacteria III. J. Cell. Comp. Physiol., **50**: 389–401.

Pratt, D. B., and G. Waddell. 1959. Adaptation of marine bacteria to growth in media lacking sodium chloride. Nature, **183**: 1208–1209.

Provasoli, L. 1958. Nutrition and ecology of protozoa and algae. Ann. Rev. Microbiol., **12**: 279–308.

Ritchie, D. 1957. Salinity optima for marine fungi affected by temperature. Amer. J. Bot., **44**: 870–874.

———. 1959. The effect of salinity and temperature on marine and other fungi from various climates. Bull. Torrey Bot. Club, **86**: 367–373.

Siepmann, R. 1959. Ein Beitrag zur saprophytischen Pilzflora des Wattes der Wesermundung II. Veröff. Inst. Meeresforsch. Bremerhaven, **6**: 283–297.

Vishniac, H. 1956. On the ecology of the lower marine fungi. Biol. Bull., **111**: 410–414.

ZoBell, C. E. 1946. Marine Microbiology. Waltham, Mass. Chronica Botanica. 240 pp.

——— and H. D. Michener. 1938. A paradox in the adaptation of marine bacteria to hypotonic solutions. Science, **87**: 328–329.

ISOLATION OF YEASTS FROM BISCAYNE BAY, FLORIDA AND ADJACENT BENTHIC AREAS[1,2]

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ABSTRACT

Investigations of the yeasts present in Biscayne Bay, Florida, have indicated the occurrence of various yeast taxa, representing 179 isolates, including species of *Saccharomyces, Hansenula, Debaryomyces, Candida, Cryptococcus, Rhodotorula, Trichosporon* and *Torulopsis.* Two asporogenous species, *Candida tropicalis* and *Rhodotorula mucilaginosa*, were the most abundant and widely distributed species in the Bay. A yeast biota, with many species similar to those isolated from Biscayne Bay, has been collected in deep sea sediments from The Bahamas. A selective enrichment culture technique has been developed, greatly facilitating the collection of yeasts from the marine environment. In general, the limited deep sea collections showed a predominance of oxidative yeasts as compared to collections made in Biscayne Bay.

The biology of yeasts in nonmarine environments has received considerable attention, but our knowledge of the yeast population present in the ocean is exceedingly meager (Fisher 1894, ZoBell and Feltham 1934, ZoBell 1946). Most of these reports, especially the earliest work, fail to describe the yeasts or yeast-like organisms so that identification is not possible, and other studies are incidental to the primary investigation of the bacterial population. Only recently have several papers appeared that deal specifically with investigations of the marine yeast biota (Bhat and Kachwalla 1955, Bhat *et al.* 1955, Novozhilova 1955).

In 1958 studies of the yeasts present in Biscayne Bay, Florida, and adjacent benthic areas were started in The Marine Laboratory of the University of Miami. This paper notes the genera and species of yeasts collected together with a brief discussion of their distribution. Further work in progress involves investigations of the possible ecological significance of yeasts and salient physiological features of selected dominant species.

MATERIALS AND METHODS

Collections in Biscayne Bay were made at 45 sites, encompassing an area from approximately 2,000 yd north of the Venetian Causeway to the southern point of Key Biscayne (Fig. 1). In general, Biscayne Bay is a shallow estuary characterized by the development of an offshore bar on a shore line of low relief.

Both sedimentary material and submerged banana stalk sections were examined. The Biscayne Bay sediments were taken with a shallow water coring device (Grein and Meyers 1958) from areas totally submerged even at low spring tide. This coring apparatus was provided with a tubular extension to permit sampling at depths to 20 ft. The banana stalk sections were submerged in area C (Fig. 1) from the end of The Marine Laboratory pier. These sections, approximately 2 in. long, were cut

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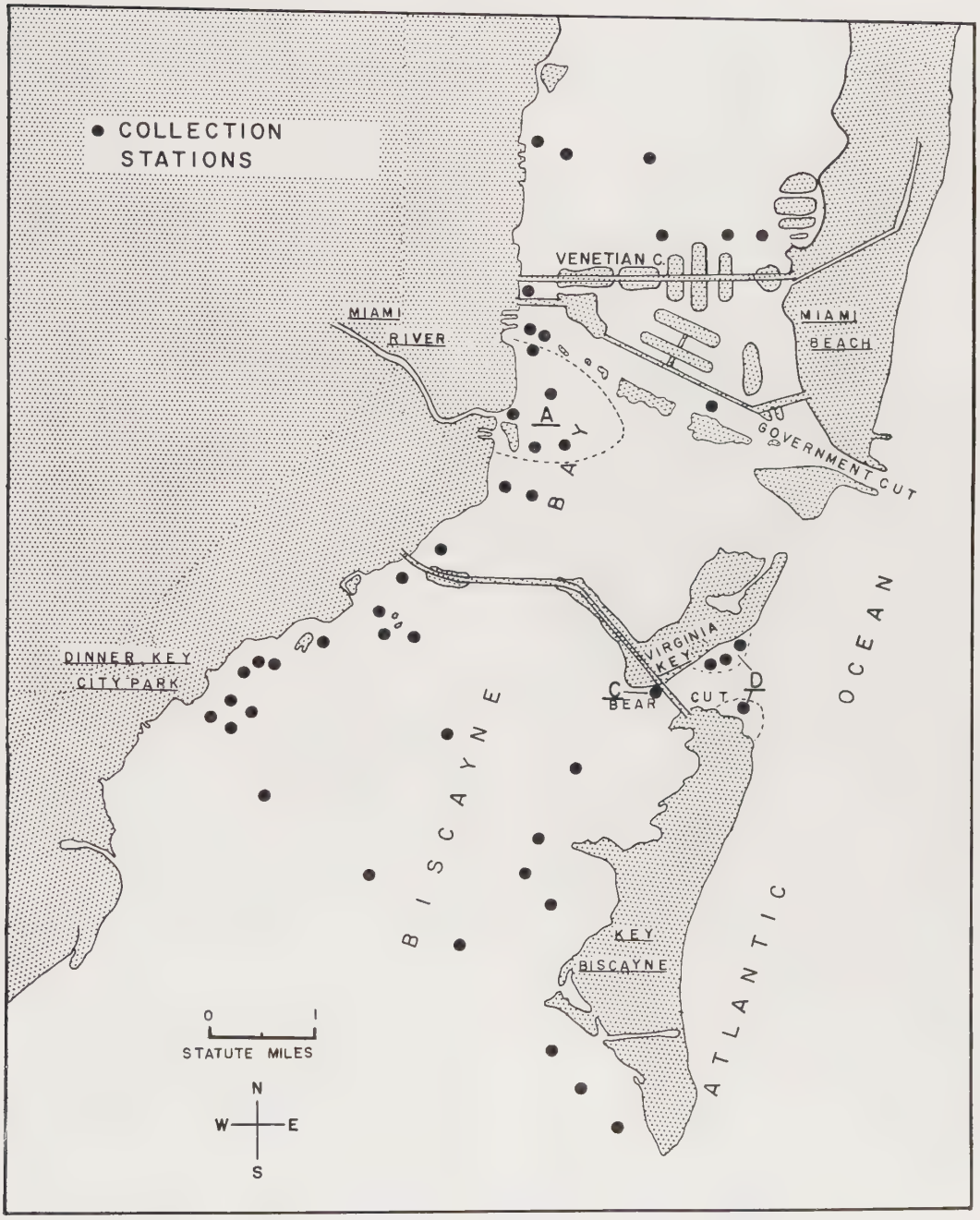

FIG. 1. Map of Biscayne Bay, Florida, with collection stations.

from nearly ripe banana stalks with a ¾ in. diameter cork borer, were sterilized (121°C for 15 min) in petri dishes, and immediately before submergence, were transferred aseptically to alcohol-sterilized plastic vials. Four to 6 vials, each with several small holes drilled at both ends to allow circulation around the banana core, were anchored along a Kordite line to ensure complete submergence at low tide. The banana stalk

sections were collected after 3 to 10 days' exposure. All sediment samples from Biscayne Bay were transported to the laboratory under refrigeration (10–15°C) and processed within 24 hr.

Deep sea sediments were collected using a gravity corer, provided with a 3-ft plastic tube liner, previously alcohol-sterilized. During transit to the collection site the tubes were sealed with sterile plugs which were removed at the time of insertion of the tube into the coring device. Immediately following collection, the tubes with the sediment core were sealed aseptically and stored in the refrigerator (6–10°C) of the vessel until subsequent examination in the laboratory. Five cores were examined from the following localities. 1) High Cay, Andros Island, The Bahamas; 500 fathoms. 2) Ten to 15 miles west of Bimini, The Bahamas; 450–455 fathoms. 3) Gun Cay Light, The Bahamas; 377 fathoms.

Three cores were taken in collection 2) and were treated as a composite sample. In collections 1) and 2), only the uppermost portion of the sediment core was examined. From collection 3), the core was divided into successive sections, each section approximately 1 cm thick. The uppermost 12 sections were used in inoculation experiments.

Sectioning was accomplished by pushing a sterile plunger through the plastic corer liner to extrude the sediment. In order to prevent possible contamination from the sides of the core immediately adjacent to the plastic liner, the material used for the inoculum was taken from the central portion of the sediment. Some of the liquid present in the tube also was used as inoculum.

Approximately 2.0 g of each separate sediment sample were used as inoculum. In tests where a submerged banana stalk section was used, the infested section was collected and aseptically transferred to a sterile blendor and ground thoroughly in 20 ml of sterile sea water. The inoculum comprised 1 ml of this suspension.

For the selective isolation of yeasts by enrichment incubation procedures, the culture medium was treated with the filter sterilized antibiotic mixture to give a final concentration of 10 mg % chlortetracycline. HCl (Aureomycin), 2 mg % chloramphenicol, and 2 mg % streptomycin sulfate. The two enrichment broths used consisted of a medium containing 2.0% glucose (Medium G), and a medium composed of 2.0% glucose, 0.1% yeast extract, and 0.5% peptone (Medium GYP). The incubation vessels consisted of 125-ml Erlenmeyer flasks, each containing 20 ml of the nutrient medium. Culture media were prepared with natural sea water as well as with distilled water. However, unless noted otherwise, all media used in the incubation and isolation procedures were prepared with sea water.

After inoculation the culture vessels were placed on a rotatory shaker (55 rpm) at the laboratory temperature of 25°C. Each flask was sampled at 24-hr intervals during the 144-hr incubation period. Full strength broth and suitable dilutions were inoculated on the surface of the primary isolation medium consisting of 2.0% glucose, 1.0% peptone, 0.1% yeast extract and 1.7% agar, prepared both with distilled water and with sea water. Replicate platings were made at each sampling. To obtain data on the approximate concentration of yeasts present in the natural environment, sediments from selected areas were inoculated directly without prior incubation enrichment, to the primary isolation medium containing the antibiotic combination noted previously. After incubation, individual yeast colonies exhibiting distinctive colonial characteristics on this medium were transferred to slants of the stock culture sea water medium of nutrient agar (Bacto) supplemented with 2.0% glucose and 0.1% yeast extract. These cultures are maintained in the microbiology culture collection of The Marine Laboratory.

To identify the organisms, the isolates were tested for carbohydrate assimilation, fermentation capacities and nitrate utilization. These tests and other diagnostic procedures used are those described by Wickerham (1951) and Lodder and Kreger-van Rij (1952). Dark pigmented species have been grouped provisionally under the gen-

TABLE 1. *Yeasts collected in Biscayne Bay, Florida*

Taxon	No. of isolates	No. of stations
Sporogenous species		
Debaryomyces kloeckeri	8	7
Hansenula anomala	9	1
Saccharomyces fructuum	2	1
Saccharomyces sp.	2	1
Asporogenous species		
Candida tropicalis	42	19
C. parapsilosis	18	10
C. guilliermondii	4	2
C. intermedia	4	1
C. boidinii	1	1
C. melinii	1	1
Cryptococcus laurentii	6	4
C. albidus	1	1
Rhodotorula mucilaginosa	24	14
R. glutinis	13	9
R. texensis	5	5
R. minuta	4	4
R. graminis	1	1
Rhodotorula sp.	1	1
Trichosporon cutaneum	10	3
Torulopsis sp.	4	1
Pullularia pullulans	2	2
"Black yeasts"	16	10

eral designation "black yeasts." The media used for identification were prepared with Difco products in distilled water according to Wickerham's formulae. Media affected by autoclaving, such as those used in the assimilation tests, were sterilized by filtration through a No. 03 Selas filter candle. All other media were sterilized at 121°C for 15 min.

RESULTS

A total of 179 isolates representing 4 sporogenous and more than 18 asporogenous species were obtained in Biscayne Bay. The species collected together with the number of isolates and stations are given in Table 1. The two most commonly isolated genera were *Candida* and *Rhodotorula,* the predominant species being *C. tropicalis, C. parapsilosis, R. mucilaginosa,* and *R. glutinis.* Except for an unidentified black yeast, only *C. tropicalis* and *R. mucilaginosa* were isolated from the vicinity of the mouth of the Miami River (Area A), a locality characterized by a rather limited yeast biota. These two species found in samples

TABLE 2. *Yeasts isolated from deep sea sediments in the Bahamas*

Cruise No. or Collection No.	Taxon	No. of isolates
(1)	*Rhodotorula mucilaginosa*	3
	R. glutinis	2
	R. texensis	1
	R. marina	1
	Debaryomyces kloeckeri	4
	Torulopsis famata	1
	Cryptococcus diffluens	1
(2)	*Torulopsis famata*	1
	Candida parapsilosis	2
	C. tenuis	2
(3)	*Cryptococcus albidus*	3
	C. neoformans var. *uniguttulatus*	2
	Candida parapsilosis	3
	C. curvata	5
	C. guilliermondii	1

taken at stations in Biscayne Bay other than in areas A, C and D were not obtained from collections in the shallow marine coastal environment of area D.

The largest variety of species was collected in area C from the banana stalk sections. This area is located adjacent to The Marine Laboratory in Bear Cut, a region subject to considerable tidal currents. A total of 17 species were isolated from the banana sections, while at the most, only 4 different species were found in sediment samples at other individual collection stations. An examination of the sediment beneath the banana traps revealed only two species, *Cryptococcus laurentii* and *Candida tropicalis.*

Samples taken from the center of the Bay showed lower numbers of yeasts and fewer species than samples collected near the shore. In general, silty muds exhibited more yeasts than sandy sediments. Periodic sampling of sediment from certain bay stations demonstrated discontinuous yeast populations.

The species of yeasts found in the deep sea sediments are listed in Table 2. While large numbers of yeasts were not found in the deep sea sediments, a considerable variety of isolates were observed. Differences were apparent in the dominant species of individual collections. In the mycological

examination of the upper 12 cm of the core from Cruise No. 3, yeasts were found only in the uppermost 2-cm layers.

The direct inoculation of selected Biscayne Bay sediments on the primary isolation medium yielded only comparatively few yeasts. Higher colony counts were obtained on this medium prepared with sea water than on a corresponding medium prepared with distilled water. In an examination of sediments from 7 different bay localities using direct plating, a maximum number of 20 yeast colonies per gram of wet sediment was noted. Frequently, further development of different species occurred upon incubation of other portions of the same inoculum in the enrichment culture broths. The antibiotics added to the latter media, as well as the antibiotics present in the primary isolation medium, permitted yeasts to develop but inhibited the growth of marine bacteria.

The 2.0% glucose medium (G) supported development of a variety of yeasts. However, selected pure cultures of actively metabolizing yeast cells, inoculated into this medium, displayed only negligible growth, although the cells remained viable over a 10-day period. It is probable that the growth of yeasts in Medium G, following inoculation of sediments or banana suspensions, is due in large part to the various metabolites in the natural substrate used as the inoculum. The addition of yeast extract and peptone to Medium G greatly increased the growth rates of individual species as well as the total yeast growth in the enrichment flasks. A more or less general succession or sequence in the development of different types of yeasts occurred during the 6-day incubation period especially in Medium G. The white or hyaline yeasts developed initially followed by the rhodotorulas. The latter reached a maximal population after approximately 96 hr. Species of black yeasts, when present, were not isolated generally until after the fifth day of incubation. The growth of species of *Rhodotorula*, especially *R. mucilaginosa*, was particularly favorable in Medium GYP. Black yeasts, found in certain samples incubated in Medium G, were not isolated along with other yeasts from the same sample in the GYP medium.

DISCUSSION

Most of the species of yeasts collected in Biscayne Bay are taxa known from nonmarine localities, and the majority of the isolates of individual species are quite similar physiologically to their terrestrial counterparts. However, the isolation of microorganisms considered terrestrial in origin from marine and brackish environments is not uncommon (Aaronson 1956, Grein and Meyers 1958, Meyers and Reynolds 1959). Of course, the mere occurrence of fungi in marine areas does not prove that the organisms are of marine origin, or conversely, that they are adventitious halotolerant terrestrial species. The criteria for determining affinity of fungi to the marine environment require much further study and evaluation before the significance of the occurrence in the sea of fungal representatives, usually considered "typically" terrestrial or nonmarine, is known.

While the occurrence of indigenous marine species of yeasts, as such, has not been demonstrated in this work, the possible existence of marine strains of particular species warrants consideration. Certain of the species, especially *Candida parapsilosis*, examined on a large number of carbon compounds have shown distinctive physiological characteristics different from those of isolates of the same species of known terrestrial origin. Further studies of these physiological differences are in progress in this laboratory.

In general, the limited deep sea collections showed a predominance of oxidative yeasts as compared to collections made in Biscayne Bay. Possibly this may be due, in large part, to the low organic content of the sediments of the open sea.

The abundance of *Candida tropicalis* in the Biscayne Bay collections agrees with the observations of Bhat and Kachwalla (1955) that this species is quite common in marine localities. However, the examinations of deep sea sediments (as well as plankton)

from the subtropical Atlantic Ocean have not revealed the presence of *C. tropicalis*, although other yeasts, including *Rhodotorula mucilaginosa*, prevalent in Biscayne Bay, have been found in deep sea material. The latter species, as well as *Cryptococcus laurentii, Candida guilliermondii, Debaryomyces kloeckeri*, and *Saccharomyces fructuum*, also were isolated by Bhat and Kachwalla in the Indian Ocean.

The large number of species isolated from the banana stalk traps may be attributed to a complexity of factors. The hydrography of the Bear Cut channel is such that tidal currents subject the banana stalk substrates to alternate flushing with bay and ocean waters, thereby permitting the establishment of fungal representatives from the respective environments. The plastic vials, containing the banana stalk cores, were heavily encrusted with fouling organisms such as the barnacle, *Balanus amphritrite*, the amphipods, *Podocercus brasiliensis* and *Erichthonius brasiliensis* and various types of algae, including *Sargassum*, entangled with the lines. It is conceivable that these communities serve as a reservoir of, or stimulate the growth of, yeast and yeast-like organisms. The association of species of *Rhodotorula* with various marine invertebrates observed in these studies suggests that this group of pigmented yeasts is well adapted for halophilic metabolic activities.

A larger spectrum of cultural techniques than those used here is necessary to obtain a more complete analysis of the different types and species of yeasts in a particular sample. The different growth rates of the various species, especially during the initial 24 hr of incubation, may account in a large part for the frequency of isolation of individual species and the patterns of succession observed. Present knowledge of the biology of marine yeasts is still too meager for a definitive evaluation of the ecological observations noted in this paper, particularly in view of the variabilities in the enrichment culture method. Work in progress in this laboratory on the metabolism of various of the marine isolates should facilitate a better understanding of the role of yeasts in the sea.

REFERENCES

Aaronson, S. 1956. A biochemical-taxonomic study of a marine micrococcus, *Gaffkya homari*, and a terrestrial counterpart. J. Gen. Microbiol., **15**: 478–484.

Bhat, J. V., and N. Kachwalla. 1955. Marine yeasts off the Indian coast. Proc. Indian Acad. Sci., Sec. B, **41**: 9–15.

———, ———, and B. N. Moody. 1955. Some aspects of the nutrition of marine yeasts and their growth. J. Sci. Ind. Res., Sec. C, **14**: 24–27.

Fisher, B. 1894. Die Bakterien des Meers nach den Untersuchungen der Plankton-Expedition unter gleichzeitiger Berücksichtigung einiger älterer und neuerer Untersuchungen. Ergebnisse der Plankton-Expedition der Humboldt-Stiftung, **4**: 1–83.

Grein, A., and S. P. Meyers. 1958. Growth characteristics and antibiotic production of actinomycetes isolated from littoral sediments and materials suspended in sea water. J. Bacteriol., **76**: 457–463.

Lodder, J., and N. J. W. Kreger van Rij. 1952. *The yeasts. A taxonomic study.* North Holland Publ. Co., Amsterdam, Holland.

———, ———. 1955. Classification and identification of yeasts, part III. Lab. Pract., **4**: 53–57.

Meyers, S. P., and E. S. Reynolds. 1959. Growth and cellulolytic activity of lignicolous deuteromycetes from marine localities. Canad. J. Microbiol., **5**: 493–503.

Novozhilova, M. I. 1955. Kolichestvennaya kharakteristika, vidovoi sostav i rasprostranenie drozhzhevykh organizmov v Chernom, Okhototskom moryrakh i v Tikhom okeane. Trudy Inst. Mikrobiol., **4**: 155–195.

Phaff, H. J., E. M. Mrak, and O. B. Williams. 1952. Yeasts isolated from shrimp. Mycologia, **44**: 431–451.

Wickerham, L. J. 1951. Taxonomy of yeasts. U. S. Dept. Agr. Tech. Bull. No. 1029, pp. 1–55.

ZoBell, C. E., and C. B. Feltham. 1934. Preliminary studies on the distribution and characteristics of marine bacteria. Bull. Scripps Inst. Oceanog. Tech. Ser., **3**: 279–296.

———. 1946. *Marine microbiology.* Chronica Botanica Co., Waltham, Mass.

PHYTOPLANKTON COMMUNITIES OF WESTERN LAKE ERIE AND THE CO_2 AND O_2 CHANGES ASSOCIATED WITH THEM

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ABSTRACT

Phytoplankton studies using samples concentrated by settling revealed population densities several times higher than those reported previously, based on centrifuge and bolting cloth concentrates. Clear and dark bottle experiments employing natural population densities revealed photosynthetic yields averaging 1.6 μmoles of CO_2 absorbed per microliter of plant volume per hour. This yield is three times higher than previously reported yields based on artificially concentrated populations. Comparison of daily photosynthetic yields per square meter of water as computed from bottle data with yields measured under completely natural conditions indicated an additional twofold discrepancy, with the natural condition yielding the higher value. The reduction of turbulence in bottles may be operating to reduce the photosynthetic yield. Eddy diffusivity in western Lake Erie, computed from pH change in the dysphotic zone, and vertical pH gradients, is about 25 cm^2/sec. Simultaneous determinations of CO_2 and O_2 changes showed that O_2 changes usually were lower than CO_2 changes, suggesting that processes other than photosynthesis are influencing the CO_2 and O_2 budgets.

During the past 30 years the aquatic communities of western Lake Erie have received rather intensive study. Until 1956, however, the phytoplankton studies utilized centrifuge and bolting cloth to concentrate the organisms before counting, and average standing crops observed were less than one microliter of plant matter per liter (Verduin 1951, 1954). In the summer of 1956 Davis (1958) used a direct count method which avoided the losses inherent in centrifuge and bolting cloth methods. His average standing crop for the summer amounted to 6 μl/L which is similar to the maximal crops reported during the previous 30 years, and is 10 times the previous averages. Much of the previous research in photosynthesis was also based on concentrated samples (Verduin 1956a). The present paper presents the results of a research program using natural population densities.

During the period June, 1957, to September, 1958, the National Science Foundation subsidized a study of phytoplankton at Put-in-Bay, Ohio, in western Lake Erie (G3836). The work was intensive in the summer months. Miss E. E. Whitwer, Associate Professor of Biology at Bowling Green State University, conducted photosynthesis investigations (clear and dark bottle) during both summers. Phytoplankton abundance was determined by Frances Piasecki Kramer during the summer of 1957, and by Mr. Fred Keefer during the summer of 1958. Mr. Bruce Cowell studied photosynthesis under completely natural conditions during the summer of 1958. Mrs. Kramer and Mr. Cowell were undergraduate students at Bowling Green State University, and Mr. Keefer at Thiel College, Greenville, Pennsylvania. One of the significant discoveries in this study was that an intelligent undergraduate student can be trained in a few days to take charge of a portion of this type of research program and carry it satisfactorily.

The facilities of the F. T. Stone Laboratory (The Ohio State University, Natural Resources Institute), Put-in-Bay, Ohio, were used in this study.

METHODS

Phytoplankton abundance was determined using a modification of the Kolkwitz method (Ruttner 1953, p. 110). A water sample was poured into a stender dish to a depth of 1 cm, 3 drops of modified Lugol's solution (10 g I_2, 20 g KI, 200 ml H_2O, 20 ml glacial acetic acid) were added to kill the

organisms and the samples were allowed to settle overnight before counting. Then 10 fields were counted, at 100× magnification, by simply immersing the 10× objective until the organisms on the bottom came into focus. The samples were also examined at 28× and 430× magnifications. If components of such a size were present that counting at one or the other of these magnifications was indicated, then 10 fields of those components were counted, and corrected estimates of their abundance were applied to the counts at 100× magnification. For example, for *Ceratium*, which has a large volume, an estimate of its volumetric abundance at 28× magnification is much more reliable than one based on the few individuals encountered at 100× magnification. Conversely, small cells of the order of 5μ in longest dimension are likely to be missed at 100× magnification, and should be counted at 430× magnification. The volumes per individual of the major components were determined by measuring 10 individuals at 430× magnification; the averages so obtained were used to compute the phytoplankton volume per liter of water. Identification of major components was made using Prescott (1951) and Tiffany and Britton (1952).

Photosynthetic and respiratory rates in bottles (35 ml) were determined by measuring pH difference between dark bottles and clear bottles, suspended at ½-m intervals in the euphotic zone, and converting to CO_2 change by referring to a differential titration curve (Verduin 1956a). Change of CO_2 under completely natural conditions was determined by measuring the pH of samples collected at intervals and computing the CO_2 change during the interval by reference to the differential titration curve. Change of O_2 under completely natural conditions was measured in similar samples using the Winkler technique. These samples were taken at approximately 4-hr intervals by day, with an 8-hr interval at night.

Light penetration measurements were made with a submarine photometer containing a Weston photronic cell.

RESULTS AND DISCUSSION

In Table 1 the major components observed during specified time intervals are presented, with average values of phytoplankton volume, of photosynthesis per liter of water per day and per microliter of plant volume per hour. The data on per liter of water per day are based on pH change in open water as determined from surface samples taken at 4-hr intervals during the day. A major component is defined as one which comprises at least 20% of the plant volume in the community. This table provides an ecologic classification of the phytoplankton communities comparable to that long used by terrestrial plant ecologists when they identify a community by its volumetrically dominant genera. Such classification of phytoplankton communities has long been obscured by the tendency to count individuals without regard to their widely discrepant volumes. The presence of the flagellates, *Chlamydomonas, Cryptomas,* and *Ceratium,* as major components of the communities is a feature never observed in the concentrated samples of previous years (Chandler 1940, 1944; Verduin 1951, 1954). It seems likely that these components escaped the concentrating and counting devices formerly utilized. The phytoplankton volumes per liter in Table 1 also are greater than those reported in previous years, and are in close agreement with the data of Davis (1958) for the summer of 1956, and with data from other aquatic habitats in northwestern Ohio (Verduin 1959b).

The photosynthetic rates computed from pH change in open water reveal several interesting features. A gradual increase in photosynthetic yield per day was observed as each summer progressed, but the increase was much smaller than the increase in phytoplankton volume. Thus a fivefold increase in phytoplankton crop during the summer of 1957 was associated with only a twofold increase in photosynthetic yield. Similarly in 1958 a sevenfold rise in phytoplankton crop was associated with only a 40% increase in photosynthetic yield. The photosynthetic yield in those bottles exposed to

TABLE 1. *Phytoplankton communities, population densities, and photosynthetic rates*

Month	Major Components	Population density (μl/L)	Photosynthetic Rate	
			open water surface sample* (μmoles CO_2 absorbed/L/day)	rate in bottles (μmoles CO_2 absorbed/μl of algae/hr)
June 1957	*Cyclotella, Fragilaria, Cryptomonas, Pediastrum*	2	60	2.1
July 1957	*Cyclotella, Pediastrum, Ceratium, Anabaena, Melosira*	4	99	1.3
Aug. 1957 Sept.	*Cyclotella, Botryococcus, Coelosphaerium, Microcystis*	10	137	1.2
Oct. 1957 Nov., Dec.	*Fragilaria, Melosira*	2		
Feb. 1958 Mar.	*Chlamydomonas, Asterionella, Tabellaria*	3		
April 1958 May	*Cyclotella, Chlamydomonas, Melosira, Glenodinium*	3		
June 1958	*Cryptomonas, Pediastrum, Melosira, Microcystis, Sphaerocystis*	1	48	4.6
July 1–15 1958	*Cyclotella, Microcystis Ceratium, Pediastrum*	3	52	2.7
July 15–30 1958	*Microcystis, Ceratium, Aphanizomenon, Cyclotella*	6	57	1.3
Aug. 1958	*Aphanizomenon, Ceratium, Melosira, Stephanodiscus, Fragilaria*	7	69	1.5

The summer data are averages of 15 or more samples. The October-May data are averages of 4 samples taken at approximately monthly intervals. The bottle data represent photosynthesis at optimal light intensity, *i.e.*, only data from bottles showing maximal yields were used, usually these were at 0.5–m depth.

* These rates represent net photosynthesis, *i.e.*, in excess of the respiration rate of the total aquatic community, the bottle data include black bottles, hence are corrected for respiration and represent gross photosynthesis.

optimal light (usually at 0.5-m depth) paralleled the open water data rather closely, and the photosynthetic yield per microliter of plant volume showed an inverse relation to the density of standing crop. Wright (1959) also observed such an inverse relationship.

In a previous paper (Verduin 1956a), I published photosynthetic yields per unit of plant volume as determined for bolting cloth concentrates of phytoplankton. These concentrates were studied at a population density of about 100 μl/L. This is similar to the density used in laboratory cultures of algae, and for years I believed that natural concentrations were too low to yield measureable photosynthetic rates. The average yields at optimal light intensity observed in such concentrates amounted to 0.5 μmoles/μl/hr. This is less than half the lowest yield appearing in Table 1. Moreover, the yields in Table 1 are similar to those reported for *Chlorella* (Verduin 1953) growing in highly enriched culture media and to the yields observed in natural communities by Wright 1959, Talling 1957, and Verduin 1959b. It seems likely that the yields in Table 1 are more nearly representative of natural rates than are those obtained with concentrated populations. However, during the summer of 1957 a naturally high population density averaging 57 μl/L was encountered on 4 consecutive days, and it exhibited an hourly photosynthetic yield of only 0.23 μmoles/μl. Thus the inverse relation between photosynthetic yield per unit plant volume and population density becomes the more evident when such extremes

TABLE 2. *Comparison of photosynthesis and respiration (μmoles CO_2 change/L/hr, in bottles) at different times of the day, during the summer of 1957*

Time	0700–1000	1100–1400	1500–1800
Photosynthesis	9.5	11.8	9.2
Respiration	–0.5*	3.4	4.6

Each value is an average of 30 or more experiments.
* The difference between this value and the other respiration values is statistically significant, beyond the 0.01 confidence limit. The other differences appearing in the table are of low statistical significance, within the 0.05 confidence limit.

are examined. The fact that a 4-day sample could differ so markedly from the summer's average also points up the low level of confidence which can be placed in studies that represent only a few days' work. The day-to-day variation encountered in our studies was so large that the lower extremes in phytoplankton crops and in photosynthetic yields per liter of water and per microliter of plant volume differed from the upper extremes by a factor of 10 or more. Rodhe (1958) has reported similar variations in yields determined by the C^{14} method.

One additional feature of Table 1 warrants discussion. The daily photosynthetic yields per liter of water during the summer of 1957 were consistently higher than those of 1958. Indeed the average for 1957 was almost twice that of 1958. This comparison shows that season-to-season variations are large and that a single season's study is a "small sample" from a statistical viewpoint. It should be emphasized that the open water yields represent "net photosynthesis" (*i.e.* in excess of community respiration). A comparison of these with gross photosynthesis in the optimally lighted bottles revealed that net photosynthesis in the surface layers was practically equal to gross photosynthesis in the bottles. These observations led us to suspect that the bottle data representing natural population densities were still unrepresentative of photosynthetic performance under completely natural conditions, and erred on the conservative side.

In a previous paper (Verduin 1957) I presented evidence of a marked reduction in photosynthetic yield during afternoon hours under completely natural conditions. This

TABLE 3. *Comparison of photosynthesis and respiration (μmoles CO_2 change/L/hr) in unsterilized and oven-sterilized bottles*

	Photosynthesis		Respiration	
	Unsterilized	Oven sterilized	Unsterilized	Oven-sterilized
1957 midday	11.8	12.1	3.4	–0.007
1958 a.m.	6.2		1.4	–0.7
1958 p.m.	5.7		2.7	1.0

Data are average values of 30 or more experiments during each summer. The respiration values in oven-sterilized bottles are significantly different (beyond the 0.01 confidence limit) from those in unsterilized bottles.

observation led us to study the photosynthetic and respiratory rates in bottles at different times of the day. The average results, at optimal light, for an entire summer's work, are presented in Table 2. It appears that the gross photosynthetic rate in bottles was not significantly lower during afternoon hours than during the morning hours, but the respiration rate in the dark bottles was notably higher during the afternoon hours than during the morning hours, when a slightly negative average was obtained. This would help to account for the reduced rates observed in net photosynthesis in open water during afternoon hours, but the questionable representativeness of black bottle respiration data (Steemann Nielsen 1955; Ryther 1954) led us to design an additional experiment.

Two series of bottles were prepared, one set of which was baked in an oven at 140°C every night, while the other was simply washed and turned upside down to drain overnight as was our routine practice. This comparison was continued throughout the entire summer of 1957, in the midday experiments. During the summer of 1958 one set of black bottles was baked nightly in this fashion but clear bottles were not. The average data at optimal light intensity for both summers are presented in Table 3. Gross photosynthesis in both sets of bottles (1957) was practically identical, but respiration in the sterilized bottles was sharply reduced. In the 1958 data, morning and afternoon results again showed essential agreement, confirming the observations in Table 2 and the sterilized dark bottles exhib-

TABLE 4. *Comparison of CO_2 and O_2 changes (μmoles/l/hr) in surface samples from western Lake Erie during the summer of 1958*

Changes observed between hours:		0600–0900	0900–1100	1100–1300	1300–1600	1600–1900	Avg. O_2/CO_2
Data for period June 24 to July 19	O_2	2.4	0.7	3.8	4.9	–2.2*	0.62
	CO_2	4.0	5.2	5.0	4.7	–0.9*	
Data for period July 20 to Aug. 25	O_2	3.7	8.3	5.7	2.9	0.7	1.2
	CO_2	3.3	7.6	4.9	4.7	–1.8	

*Negative O_2 change represents oxygen consumption, negative CO_2 change represents CO_2 evolution. Samples were collected daily, and data represent averages for the period indicated.

ited much lower respiration rates than the unsterilized ones. The discrepancy persisted in the afternoon experiments although no baking intervened between the morning and afternoon tests. These data suggest that respiration rates observed in dark bottles are probably not representative of respiration rates under natural conditions, but the artificial respiration rate which develops in the black bottle is matched by a similar respiration rate in the clear bottles thus causing the estimates of gross photosynthesis to agree. This agreement indicates that the organisms responsible for the high respiration rate in the unsterilized bottles are not inhibited significantly by the light which penetrates the clear bottles, in contrast to Steemann Nielsen's (1955) finding that inhibition of bacterial respiration occurred in the clear bottles.

The experiments with bottles at several depths, when correlated with light penetration data, revealed the relation of photosynthetic rates to light intensity. A comparison of these data with curves previously published for concentrated populations (Verduin 1956a) and with curves published by other investigations of phytoplankton photosynthesis (Ryther 1954, Manning and Juday 1941, Ichimura 1957) indicated no significant divergence in curve shape in our experiments at natural population density. Photosynthetic rates rose sharply between zero and 30% of full sunlight, remained essentially constant between 30 and 80%, and showed reduced rates (by almost 20%) in the surface bottles. Although the natural population density resulted in much higher yields per unit of plant volume, the shape of the curve of photosynthesis against light intensity was not altered. Consequently graphs of this relationship are not included in the present report, because they only serve to confirm those already abundantly represented in the literature.

During the summer of 1958, the pH and O_2 content of surface samples taken from the bay at approximately 3-hr intervals was used to compute CO_2 and O_2 change during these intervals. The averaged results appear in Table 4. Because a distinct change in community composition occurred during the third week of July the data were divided into two sets: June 10 to July 19, and July 20 to August 27. During the first period CO_2 change usually exceeded O_2 change, with an average O_2/CO_2 ratio of 0.62 for the period. This is similar to the ratios observed by Park *et al.* (1958) in shallow waters of Texas bays. But during the second period the average ratio was 1.2.

In the above presentation allusion has been made to the probable unrepresentativeness of bottle data. The observation that pH change in surface samples during a 3-hr interval was practically equal to the difference between the optimally lighted clear bottle and the black bottle, was primarily responsible for the suspicion. Students of photosynthetic yield have usually based

TABLE 5. *CO_2 change as computed from pH change under completely natural conditions*

Time 0600–1100					
	pH Time		ΔCO_2 milli	m^3*	ΔCO_2 for
Depth (m)	0600	1100	-moles/m^3	represented in water column	entire water column
0.1	8.24	8.42	24	1	24
1.0	8.23	8.40	24	1	24
3.0	8.21	8.35	19	2	38
5.0	8.20	8.31	15	2	30
8.0	8.19	8.28	12	3	36
					152 millimoles/m^2

* The first two samples are considered representative of the first 2 m, the 3- and 5-m samples of the next 2-m intervals, the 8-m sample of the last 3 m in the water column.

computations per unit of water area on bottle data (Verduin 1956b, Ryther 1956, Manning and Juday 1941). In such computations the average yield per liter from a series of bottles suspended in the euphotic zone is regarded as representative of the yield in nature and when multiplied by appropriate factors for euphotic zone depth and length of day an estimate of yield per unit area is obtained. However, if yields in bottles are not representative of those in nature the estimates may contain a considerable error. Therefore, an experiment was designed to measure pH change at several depths and at 4-hr intervals to provide estimates of CO_2 change in the entire water column throughout the day.

Samples were taken from five depths (0.1, 1, 3, 5 and 8 m) at a station in the channel between South Bass and Middle Bass islands where the water was 9 m deep. The samples were returned to the laboratory for pH determination. Table 5 shows an example of the data obtained and computation of the CO_2 change for the water column.

In this study no corrections were applied for CO_2 exchange between air and water, as the magnitude of such exchange is considered negligible in comparison to the CO_2 changes within the water (Verduin 1959a, 1959b).

The computations in Table 5 also assume that the pH changes due to exchange of water between sampling times will not introduce a systematic error. Random errors will, of course, be introduced by this influence, and the rate computed from a single day's work may be too large or small depending on whether change of water mass increased, or decreased, the pH value. In the average from several days' work, however, such random errors will cancel.

The photosynthetic rate computed from diurnal pH change represents net photosynthesis (*i.e.* in excess of respiration by the entire aquatic community). Because nocturnal CO_2 production is practically equal to the diurnal net photosynthesis (the 0630 pH value, for example, shows no consistent trend from day to day) it is evident that the true diurnal photosynthetic rate must be twice as high as the net rate, because diurnal respiration of the entire community must proceed at a rate not less than the nocturnal, although masked by photosynthesis. Diurnal respiration of the plant component may well be higher than their nocturnal rate, hence the estimate of diurnal respiration may err on the conservative side. To compare total photosynthesis under completely natural conditions with yields estimated from bottle data, the net rates, computed from data similar to those in Table 5, were doubled. The comparison is presented in Table 6. The yields measured under completely natural conditions are more than twice as high as those computed from clear and dark bottle data. It seems likely that the enclosing of phytoplankton in bottles changes the environment greatly and causes marked reduction in photosynthetic activity. The most obvious difference in environment is the reduction in turbulence, which would tend to reduce the rate of CO_2 transport to

TABLE 6. *Photosynthetic yields (millimoles/m²/day) under completely natural conditions compared with yields computed from clear and dark bottle data*

Time interval	Photosynthetic Rates	
	Completely natural	Bottle data
June 17–July 18	450	187*
July 19–Aug. 22	576	242

* A sample computation from bottle data is made as follows:

4.8 millimoles/ m^3/hr	×	0.65 ratio of optimal rate to average in euphotic zone	×	5 depth of euphotic zone (m)	×	12 hours of daylight

The bottle data represent experiments paralleling the study under completely natural conditions; each value in the Table represents 14 days' work.

phytoplankton cells (Verduin 1959a). But the flagellate component of a community may suffer injury on the glass surface, when swimming unidirectionally in response to light stimuli, and the multiplication of a bacterial population may produce inhibitory substances. In any case it seems obvious that reliable estimates of photosynthetic rates in nature cannot be obtained by extrapolation of rates observed in bottles to the natural condition. This statement also applies to rates measured with the C^{14} method now being adopted by many investigators.

Frey and Stahl (1958) presented data for two arctic lakes showing fairly close agreement between three types of bottle data: C^{14} method, pH method, and O_2 method. In computing total inorganic carbon in the C^{14} method, they assumed that each calcium ion was associated with only one carbon atom instead of two which are present in bicarbonate. Correcting for this error the true yields with the C^{14} method were about twice as high as they reported indicating that rather large discrepancies may occur between C^{14} data and other methods. But in one lake they also obtained pH changes under completely natural conditions, and if these are used to compute photosynthetic yields the observed yield is about six times higher than the yields observed in bottles. Their paper represents only two days' experiments. Ryther *et al.* (1958) present data from two days' work in Senix Creek which show that photosynthesis based on O_2 change under completely natural conditions exceeded that observed in bottles by about 10% (average for the two days) and exceeded that computed from chlorophyll concentrations by about 25%. The study was made in extremely quiet water. The yield per m^2 per day amounted to 450 millimoles of O_2 produced, which is similar to the values appearing in Table 6. They published curves showing pH change; but they did not obtain differential titration data which would make a computation of CO_2 change possible. The plateau in their pH curves is much wider than in the oxygen curve suggesting that an O_2:CO_2 discrepancy similar to that reported above for our June–July data, and similar to that found by Park *et al.* (1958) was present. Ryther *et al.* also concluded that O_2 and CO_2 exchange between air and water were negligible.

The importance of turbulence in the aquatic environment necessitates some quantitative measure of it. The eddy diffusivity is such a parameter. Because the waters of western Lake Erie show no consistent vertical trend in temperature characteristics the usual method of determining eddy diffusivity, by solving a heat transport equation, has never been applied. The study of photosynthesis under completely natural conditions described above provided data from which computations of eddy diffusivity could be made.

Light penetration measurements revealed that less than 1% of surface light penetrated beyond 5-m depths. Hence the lower 4 m in the water column were not likely to support photosynthesis in excess of respiration, and any rise in pH observed there could be attributed to transport of the products of photosynthesis from the euphotic zone. Moreover the vertical pH gradient proved large enough to be easily detectable with the Beckman meter, so all the essential parameters for the solution of a transport equation were available, making the computation of diffusivity possible.

Table 7 presents a sample of the data used. Note that at 0600 the pH was practically uniform from top to bottom, but by

TABLE 7. *pH values at different times and depths on August 18, 1958*

Depth (m)	pH at			
	0600	1100	1500	1830
0.1	8.23	8.50	8.60	8.80
1.0	8.23	8.38	8.59	8.79
3.0	8.23	8.38	8.53	8.76
5.0	8.21	8.36	8.50	8.71
8.0	8.20	8.34	8.42	8.65

1100 a pronounced vertical gradient had developed. As the day progressed a distinct rise in pH occurred at 8 m, which must be attributed to downward transport of water of high pH, and *vice versa*. To compute eddy diffusivity from such data the 5-m level is regarded as the horizontal section across which transport occurs, and it is assumed that all the CO_2 change in the 4-m column below this level represents transport. Thus between 1100 and 1500 the CO_2 transport/cm^2/sec represented by the pH change from 8.34 to 8.42 is:

$$0.08 \times 121 \times 10^{-3} \times 400/(4 \times 3600) = 0.27 \times 10^{-3}$$

(pH change) (μmoles CO_2 change per pH unit per ml) (ml below 1 cm^2) (sec in 4 hr) (μmoles/cm^2/sec)

It is assumed that the gradient between 3 and 5 m provides an acceptable estimate of the gradient responsible for the transport. This amounts to 0.025 pH units over a 200 cm distance (average of 1100 and 1500 values). In terms of CO_2 gradient this is $0.025 \times 121 \times 10^{-3}/200 = 1.5 \times 10^{-5}$ μmoles/cm^4. The eddy diffusivity then amounts to 0.27×10^{-3} μmoles/cm^2/sec/$(1.5 \times 10^{-5}$ μmoles/cm$^4) = 18$ cm^2/sec. Between 1500 and 1830 a similar computation yields a transport of $0.23 \times 121 \times 10^{-3} \times 400/(3.5 \times 3600) = 0.88 \times 10^{-3}$ μmoles/cm^2/sec with a gradient of $(0.04 \times 121 \times 10^{-3}/200) = 2.4 \times 10^{-5}$ μmoles/cm^4. The eddy diffusivity amounts to $0.88 \times 10^{-3}/(2.4 \times 10^{-5}) = 37$ cm^2/sec. The average value for 28 days' study during the summer of 1958 was 25 cm^2/sec. This relatively high value reflects the stirring influence of the seiches. Such a computation is applicable only in waters having their euphotic zone depth less than the depth of the epilimnion.

In a previous paper (Verduin 1956b) I stated that the photosynthetic rate per unit of plant matter was one of the least reliable values in the computation of photosynthetic yields in nature. The rate of absorption of CO_2, 0.5 μmoles/μl/hr, reported at that time (based on concentrated samples) was in close agreement with data from Wisconsin lakes and from marine situations. The bottle data in Table 1, however, indicate much higher rates, averaging 1.6 μmoles/μl/hr. The comparison of bottle data and completely natural conditions (Table 6) suggests that the true photosynthetic rate must be twice as high as the bottle data would indicate, or about 3 μmoles/μl/hr. This represents an upward revision of sixfold in our estimate of photosynthetic capacity. If the inverse relation between photosynthetic rate per unit of plant matter, and population density, is taken into account it is conceivable that populations below a density of 1 μl/L may have photosynthetic capacities similar to 6–8 μmoles of CO_2 absorption/μl/hr.

The data presented here, and those of other investigators reviewed here, indicate that there are rather large discrepancies among photosynthetic yields obtained by different methods. The C^{14} method in some situations yields values about twice as high as the pH method of determining CO_2 change, the latter in many situations indicates photosynthetic yields about twice as high as the O_2 change data indicate, and the difference between bottle data and completely natural conditions may differ by a factor of two, or more, with the natural condition yielding the higher values.

The discrepancy between CO_2 and O_2 change (Table 4) presents a most interesting problem. It may be that during the exponential growth phase of a community syntheses of organic acids utilize considerable amounts of CO_2 (carboxylation) above the quantities used in glucose production, and that this process accounts for the low ratio of O_2:CO_2 change. A community in the plateau or decay portion of the population cycle may utilize much less CO_2 in such processes and would then exhibit an O_2:CO_2

ratio close to unity, as is evident in the second period of Table 4. On the other hand there may be oxygen consuming processes, other than respiration, which obscure the true oxygen yield in photosynthesis. The fact that a low O_2:CO_2 ratio is frequently observed in natural communities is well established, but the processes contributing to it warrant more intensive study.

The discrepancy between bottle data and the data obtained under completely natural conditions is equally interesting. If turbulence is important in causing this discrepancy, then a study of the magnitude of the discrepancy in waters of various turbulence should provide a test of the hypothesis. Such studies must be made, and quantitative measurements of turbulence must be included. If the discrepancy in Lake Erie, having an eddy diffusivity of the order of 25 cm^2/sec, is approximately twofold, then bottle data for marine situations, in which turbulence is of the same order of magnitude, may be similarly unrepresentative of the completely natural condition. The determination of photosynthetic rates under completely natural conditions must be extended to many more habitats before the reliability of present estimates of photosynthetic rates per unit area can be assessed.

REFERENCES

Chandler, D. C. 1940. Limnological studies of western Lake Erie. I. Plankton and certain physical-chemical data of the Bass Islands region, from September 1938, to November 1939. Ohio Jour. Sci., **40**: 291–336.

———. 1944. IV. Relation of limnological and meteorological conditions to the production of phytoplankton in 1942. Ecological Monog., **15**: 435–456.

Davis, Charles C. 1958. An approach to some problems of secondary production in the western Lake Erie region. Limnol. Oceanogr., **3**: 15–28.

Frey, D. G., and J. B. Stahl. 1958. Measurements of primary production on Southampton Island in the Canadian Arctic. Limnol. Oceanogr., **3**: 215–221.

Ichimura, Shun-ei. 1957. On the photosynthesis of natural phytoplankton under field conditions. Botanical Magazine, Tokyo, **71**: 110–116.

Manning, W. M., and R. E. Juday. 1941. The chlorophyl content and productivity of some lakes in northeastern Wisconsin. Trans. Wisc. Acad. Sci. Arts Lett., **33**: 363–393.

Park, K., D. W. Hood, and H. T. Odum. 1958. Diurnal pH variation in Texas bays, and its application to primary production estimation. Publ. Inst. Mar. Sci. Univ. Texas, **5**: 47–64.

Prescott, G. W. 1951. Algae of the western Great Lakes area. Cranbrook Inst. of Sci. Bull. No. 31, Bloomfield Hills, Mich., xiii + 946 pp.

Rodhe, W. 1958. The primary production in lakes: some results and restrictions of the 14C method. Rapp. et Proc.-verb., Cons. Internat. Explor. Mer., **144**: 122–128.

Ruttner, F. 1953. Fundamentals of Limnology (Transl. by D. G. Frey and F. E. J. Fry). Univ. of Toronto Press, xi + 242 pp.

Ryther, J. H. 1954. The ratio of photosynthesis to respiration in marine plankton algae and its effect upon the measurement of productivity. Deep Sea Res., **2**: 134–139.

———. 1956. Photosynthesis in the ocean as a function of light intensity. Limnol. Oceanogr., **1**: 61–70.

———, C. S. Yentsch, E. M. Hulburt, and R. F. Vaccaro. 1958. The dynamics of a diatom bloom. Biol. Bull., **115**: 257–268.

Steemann Nielsen, E. 1955. An effect of antibiotics produced by plankton algae. Nature, **176**: 553.

Talling, J. F. 1957. Diurnal changes of stratification and photosynthesis in some tropical African waters. Proc. Roy. Soc., B., **147**: 57–83.

Tiffany, L. H., and M. E. Britton. 1952. The algae of Illinois. Univ. of Chicago Press, ix + 407 pp.

Verduin, Jacob. 1951. Comparison of spring diatom crops of western Lake Erie in 1949 and 1950. Ecology, **32**: 662–668.

———. 1953. A table of photosynthetic rates under optimal near-natural conditions. Amer. Jour. Bot., **40**: 675–679.

———. 1954. Phytoplankton and turbidity in western Lake Erie. Ecology, **35**: 501–561.

———. 1956a. Energy fixation and utilization by natural communities in western Lake Erie. Ecology, **37**: 40–50.

———. 1956b. Primary production in lakes. Limnol. Oceanogr., **1**: 85–91.

———. 1957. Daytime variations in phytoplankton photosynthesis. Limnol. Oceanogr., **2**: 333–336.

———. 1959a. Maximal photosynthetic rates in nature. Science, **130**: 268–269.

———. 1959b. Photosynthesis by aquatic communities in northwestern Ohio. Ecology, **40**: 377–383.

Wright, John C. 1959. Limnology of Canyon Ferry Reservoir. II. Phytoplankton standing crop and primary production. Limnol. Oceanogr., **4**: 235–245.

INTERTIDAL ZONATION IN POPULATIONS OF *MYA ARENARIA*[1]

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ABSTRACT

Observations over a 2-year period on the distribution of two successive year-classes of *Mya arenaria* in a tidal area near Quincy, Mass., indicated that young clams 2–15 mm in length were subject to appreciable changes in location in intertidal areas. Such changes involved a shoreward displacement of the members of the population as they exceeded 5 mm in length, with a marked tendency for successive populations ultimately to become concentrated near an abrupt change in the beach profile.

The similarities between the patterns of clam and surface sediment distribution in this area are discussed, and it is suggested that, as in the case of sediment particles, hydrodynamic forces are primarily responsible for the zonation of *Mya* populations in intertidal areas.

Interspecific zonation of marine organisms in the intertidal zone is a frequently observed phenomenon and has been described in the case of both rocky shorelines (Stephenson and Stephenson 1949) and sandy beaches (Stephen 1929). Intraspecific zonation, in which the members of a population appear to be distributed according to size between the tide lines, has also been reported, particularly with respect to bivalve populations. Species reported as exhibiting this type of zonation include *Tellina tenuis* (Stephen 1928), *Tivela stultorum* (Weymouth 1923), and *Amphidisma ventricosum* (Rapson 1952).

Turner (1951) described the zonation of a population of soft-shell clams, *Mya arenaria*, during the different stages in growth and suggested the possibility of hydrodynamic concentration. In his opinion, clams, when dislodged from the sediment by wave action, would be sorted out by water turbulence in very much the same fashion as sediment particles, with larger members of the population deposited on the foreshore and the smaller members returned seaward in the backwash. Turner suggested that this mechanism might account for the intraspecific zonation of many intertidal species.

[1] Adapted from data contained in a thesis in biology submitted to the Graduate School of Arts and Sciences of Harvard University in partial fulfillment of the degree of Doctor of Philosophy.

The purpose of this investigation was to study the distribution of a *Mya* population over a tidal flat and to attempt to relate these observations, conducted over a two-year period, to the gross geological characteristics of the area. It was hoped that these observations might give some indication as to whether, and to what extent, the "wandering" behavior of juvenile *Mya* described by Smith (1955) accounts for appreciable changes in location of entire populations.

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METHODS

The section of tidal flat where this investigation took place is located in the Hough's Neck area of Quincy, Mass. The shoreline is characterized by a sand beach with a 5° slope that extends seaward a distance of 30 m beyond a concrete sea-wall, the base of which marks the mean high tide mark. Seaward of this 30-m point the beach levels distinctly, becoming a broad sand-mud flat with a 1° slope that extends well beyond spring low tide mark (Fig. 1). The mean

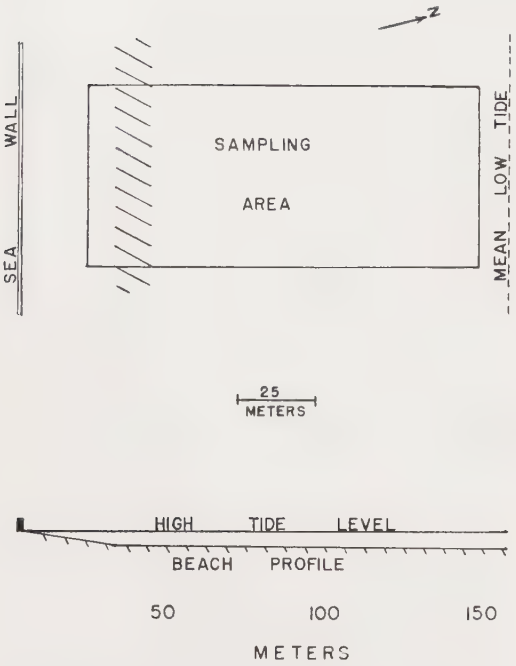

FIG. 1. Rectangular sampling area, with hatched area indicating zone of concentration of *Mya* populations, (above), and (below) beach profile showing change in slope at 30-m point.

low tide mark is approximately 175 m from the base of the sea-wall.

In August, 1956, a preliminary study was made of the distribution of *Mya* in this area. The population, with the exception of a small number of clams less than 5 mm in length that were scattered over the flats, was concentrated in a narrow zone, 15 m in width and parallel to the sea-wall. The upper margin of this zone was marked by the break in the beach profile (Fig. 1). This section of the flat was populated by members of at least three different year-classes—1953, 1954 and 1955—and the abundance of shells beneath the surface of the sediment suggested that previous year-classes may have tended to concentrate here.

In November, 1956, large numbers of clams 2–3 mm in length, the result of a late summer spawning, were observed on the flats. A sampling system was devised which would provide adequate coverage of a representative section of the total area. This section, or sampling area, was rectangular in shape, with its shoreward margin running parallel to the sea-wall 20 m seaward of its base (Fig. 1). Commencing at the 20-m point, samples were taken every 10 m along 8 separate traverses that ran parallel to each other, 10 m apart, and that extended to a point 150 m seaward of the sea-wall. A total of 112 samples within the margins of the sampling area were thereby obtained.

Sampling consisted of sieving sections of surface sediment 25 × 25 cm in surface area and 5 cm deep through wire screening with a mesh of approximately 1 × 1.5 mm. Each sample retained by the screen was returned to the laboratory and allowed to dry. The samples were then placed in a saturated solution of zinc chloride and stirred. The density of this solution was such that any clams contained in the sample would rise to the surface, free of the denser sand grains and pebbles that otherwise made detection of the smaller clams a difficult and laborious task. All the clams obtained from each sample were measured to the nearest millimeter with vernier calipers.

This sampling procedure was followed in November, 1956, and April and June of 1957. Only a superficial survey was possible in August, 1956. During the fall of 1957 these flats were covered by a heavy bed of alga, *Enteromorpha*, which prevented a complete survey. It was possible, however, to take samples along a single traverse during the fall on 3 occasions, and these samples were analyzed in the manner described above. Small samples of surface sediment, 65 cm^2 in surface area and 0.5 cm deep, were collected simultaneously. These samples were analyzed for median sediment diameter in Tyler sieves mounted on an electric-powered shaker.

By January, 1958, the *Enteromorpha* had disappeared from the flats, permitting 4 complete surveys of the sampling area during the winter and spring.

During the fall of 1956, wooden trays, 47 × 30 × 10 cm deep, were filled with clam-free sand and were sunk flush in the sediment of the flats at a position 50 m seaward of the base of the sea-wall. A section of plastic screening was attached along the

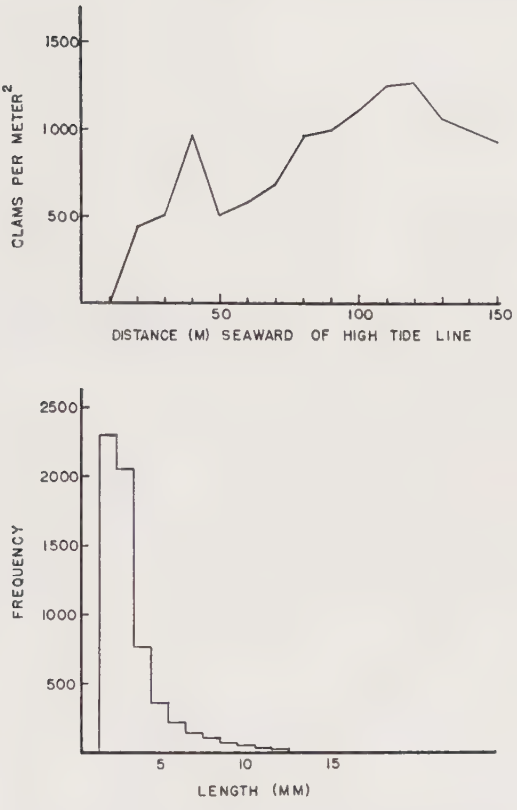

FIG. 2. Distribution (above) and size-frequency histogram (below) of 1956 year-class in November, 1956.

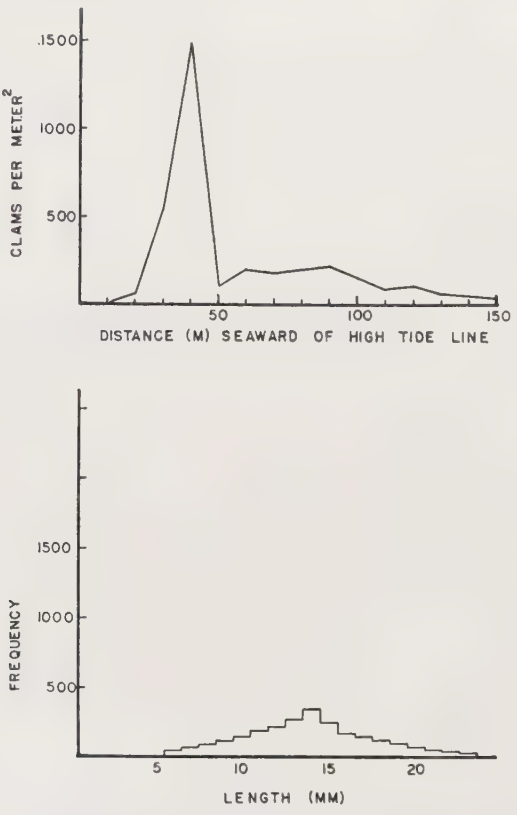

FIG. 3. Distribution (above) and size-frequency histogram (below) of 1956 year-class in June, 1957.

shoreward edge of the tray and supported in such a way as to form a vertical baffle 25 cm in height above the surface of the sediment. It was presumed that moving clams, upon coming in contact with the screening, and therefore prevented from advancing further, would bury themselves in the sediment within the tray. The sediment was examined periodically for the clams.

This experiment was repeated in the spring of 1957, but in this case the vertical screening divided the tray into a shoreward and seaward half. It was therefore possible to determine not only the size of the clams that were moving but also the direction in which the majority of the clams were moving.

The settling velocity of *Mya* of different sizes was determined by timing the rate of descent of individual live clams through a 50-cm column of salt water at 20°C. For each size group, *i.e.*, 2–4 mm, 5–7 mm, etc., the settling velocity of 20 individual clams was measured and the values averaged.

RESULTS

In November, 1956, the majority of the members of the 1956 year-class were located a considerable distance seaward of the zone inhabited by all previous year-classes (*i.e.*, 1953, 1954, and 1955). The average number of clams per m^2 of surface area, based on 8 separate traverses, at 14 different locations between the tide lines and the size-frequency distribution of this year-class are given in Figure 2.

By April, 1957, the mean size of this year-class had increased from 3.4 mm in November to 4.9 mm. Although a slightly greater proportion of clams were located in the 30- to 40-m zone than had been the case in November, the majority were again observed to be located seaward of this zone.

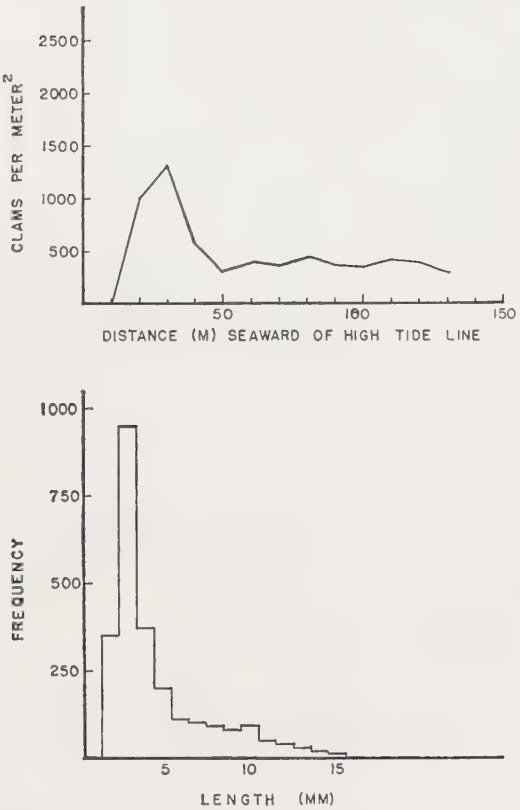

FIG. 4. Distribution (above) and size-frequency histogram (below) of 1957 year-class in January, 1958.

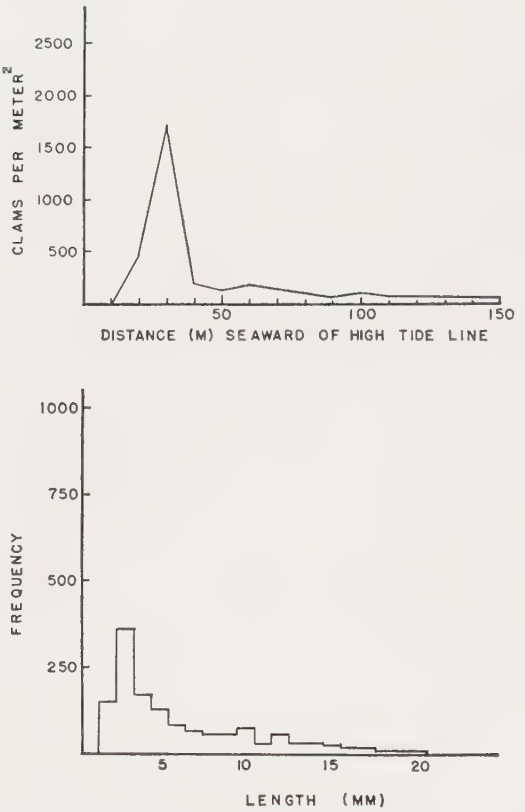

FIG. 5. Distribution (above) and size-frequency histogram (below) of 1957 year-class in April, 1958.

In June, the distribution of this year-class was observed to have undergone a striking change since April. As indicated in Figure 3, the great majority of the clams were now concentrated in the 30- to 40-m zone, with only a very small number remaining seaward of this zone. The average length was found to be 13.6 mm, indicating rapid growth during the period April–June.

By August, no clams whatsoever were to be found seaward of the 30- to 40-m zone. In effect, all of the surviving members of the 1956 year-class were now concentrated in the same zone inhabited by previous year-classes.

The results of investigations on the distribution of the 1957 year-class in the same area in January and April of 1958 are given in Figures 4 and 5. In this case the majority of clams were consistently found to be located in the shoreward portion of the sampling area. However, as growth rate accelerated during the early spring, it was again observed that clams tended to disappear from the seaward portion of the sampling area and to concentrate in that region of the foreshore, marked by the change in beach profile, which was populated by members of previous year-classes.

The combined results of the tray experiments conducted during the spring of 1957 are presented in Figure 6. No clams in excess of 15 mm in length were ever found in the trays, which suggests that clams that have attained this size are essentially stable with respect to intertidal location. It was also noted that considerably more clams were moving shoreward than seaward. Finally, it was observed that clams 2 and 3 mm in length were never particularly abun-

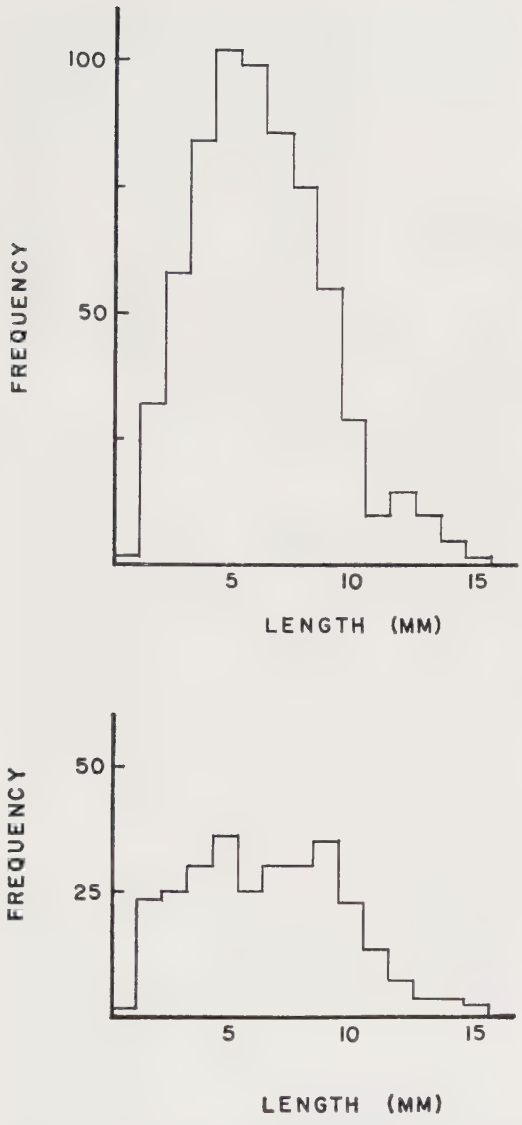

FIG. 6. Size-frequency distribution of clams moving shoreward (above) and seaward (below) during spring, 1957.

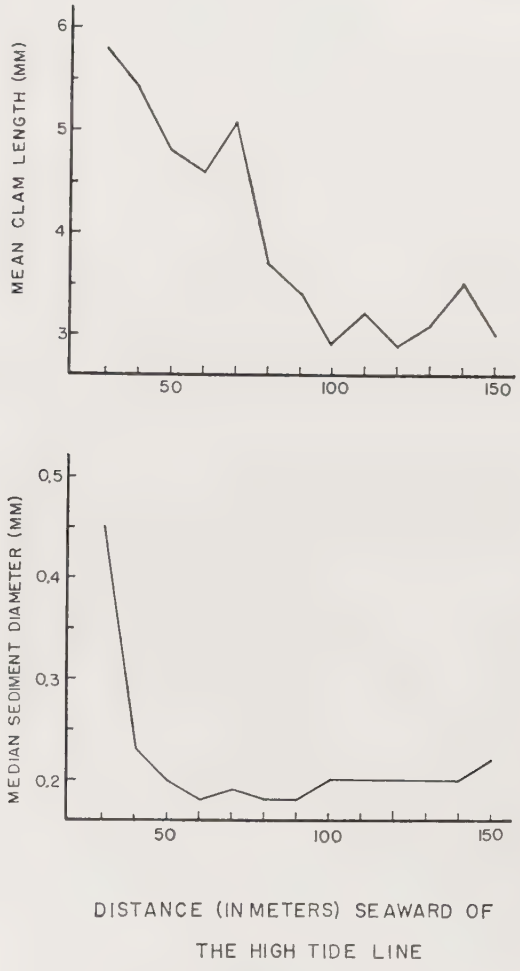

FIG. 7. Mean clam length (above) and median sediment diameter (below) at different locations in sampling area during fall of 1957.

dant in the trays, even though they were more abundant on the flats during these experiments than clams of larger size.

In Figure 7 the results of the three separate traverses are averaged with respect to mean clam length and median sediment diameter at different locations in the sampling area. The trend for both to decrease in a seaward direction is apparent. In Figure 8, the settling velocities of different-sized clams are contrasted with those of sand grains.

Data on the size and degree of sorting of clam samples taken at different times and at different locations in the sampling area are presented in Table 1. Degree of sorting, or sorting coefficient, was determined by the formula employed by Miller and Zeigler (1958):

Degree of Sorting (D. S.) $= P_{80} - P_{20}/P_{50}$

where P_{50} = median length

P_{80} = the length exceeded by 20% of the sample

P_{20} = the length exceeded by 80% of the sample

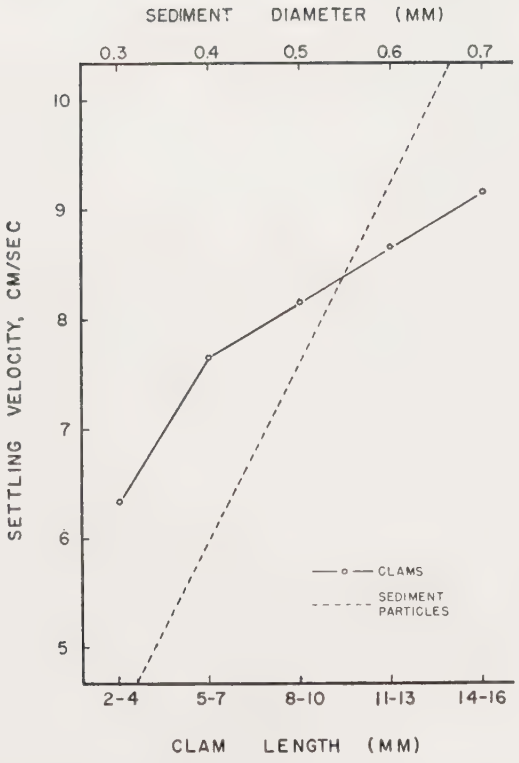

FIG. 8. Settling velocities of clams and sediment particles of different sizes.

Obviously, the larger the value of the sorting coefficient, the poorer the sorting, or the greater the variation in particle size in the sample.

DISCUSSION

Numerous studies have been made upon the transport of sediment particles by waves, including analyses of particle motion and displacement in wave tanks (Rector 1954; Ippen and Eagleson 1955; Manohar 1955; Eagleson, Dean and Peralta 1957) and observations on the pattern of sediment distribution under natural conditions (Miller and Zeigler 1958). On the basis of these investigations it is possible to make the following generalizations: 1) the depth at which particles are in equilibrium, undergoing no net displacement either shoreward or seaward, varies indirectly with settling velocity and therefore with size, 2) when particles are thrown into suspension under a shoaling wave, the coarser fractions settle out most rapidly and experience a net displacement shoreward; the finer fractions remain in suspension longer, moving seaward under the trough and shoreward under the crest, with a net displacement seaward, 3) along a line normal to wave direction, median sediment diameter increases in a shoreward direction, with the largest particles tending to accumulate in that zone characterized by a break in the beach profile involving an increase in slope, 4) only the finer particles under a breaking wave will be able to pass shoreward of that point where an increase in beach slope occurs, 5) the degree of sorting decreases in a shoreward direction up to the break in the beach profile, sorting being poorest at this point; sorting steadily improves shoreward of this point.

Clams obviously are dissimilar to sediment particles in shape, size and specific gravity, all of which are factors that tend to determine the sorting characteristics of any

TABLE 1. *Mean clam length and degree of sorting (D. S.) of clam samples taken at 3 different locations in sampling area near Quincy during period November, 1956–May, 1958.*

Date	Distance from high tide mark (m)	Mean clam length (mm)	D.S. of clam sample
Nov., 1956	20	2.6	.33
	30	3.9	1.2
	100	3.3	.33
Apr., 1957	20	3.0	.7
	30	5.3	.9
	100	4.4	.7
June, 1957	20	2.8	
	30	10.2	
	100	11.8	
Feb., 1958	20	3.6	.4
	30	3.9	1.0
	100	3.8	.5
March, 1958	20	3.6	.4
	30	4.2	.9
	100	3.8	.6
April, 1958	20	3.6	.7
	30	3.3	1.0
	100	5.6	.5
May, 1958	20	3.8	.8
	30	4.4	1.2
	100	5.0	.8

particle. Also, clams, if dislodged, have the potential ability of digging back into the substratum and may therefore exercise some control over their distribution, unlike inert sediment particles. However, settling velocity, as explained above, is an important criterion with respect to the location of particles in the region of shoaling and breaking waves. Therefore it would seem safe to assume that, once both clams and sediment particles have been set in motion by wave action, where they come to rest will depend to a certain degree on size. In this respect their individual patterns of distribution, though mutually independent one from the other, should be governed by the same hydrodynamic forces.

The pattern of clam distribution in the sampling area agrees in general with the principles of sediment sorting by shoaling waves described above. Throughout the two-year period of investigations, the great majority of the larger members of the population—clams in excess of 5 mm in length—were invariably found to be concentrated in the 30- to 40-m zone, at the abrupt change in beach slope. Seaward of this zone, as well as shoreward, the population was most typically represented by clams of smaller size. Similarly, the degree of sorting of clam samples obtained in the 30- to 40-m zone was invariably poorer than at stations either seaward or shoreward of this area.

The following line of reasoning is therefore offered as an explanation for the observed movements of the 1956 and 1957 *Mya* year-classes in the sampling area. In the case of the former year-class, the juvenile clams that had set in the late summer in the seaward portion of the sampling area grew very slowly during the fall and winter. As long as these individuals remained small, they experienced no net horizontal displacement shoreward when dislodged from the substratum by wave action but, when thrown into suspension with the fine sediment particles, they simply oscillated to and fro under the trough and crest of each wave. The tray experiments strongly suggest that, despite their numerical superiority, clams in the 2–3 mm size range comprised a relatively small percentage of those that were being displaced either shoreward or seaward. As the members of this year-class began to grow quite rapidly during the spring months, however, their sorting characteristics changed. Once dislodged from the substratum, they would experience a net horizontal displacement shoreward under each succeeding wave, and, like the coarser sediment particles, eventually be carried to the break in the beach profile. Further shoreward progress would at this point be retarded by the combined effects of gravity and reduced rate of tidal advance, permitting the uninjured clams to bury in this zone and avoid further movement. In this manner all the members of this year-class eventually became concentrated in this zone.

In the case of the 1957 year-class, the situation was initially quite different in that spatfall was apparently heaviest in the shoreward portion of the sampling area. However, the tendency for the clams located in the seaward portion eventually to disappear and for all the members of the year-class to become concentrated ultimately in the 30- to 40-m zone was again apparent.

It would seem unlikely that clams are dislodged from the substratum and transported by wave action under average sea conditions in this area. During periods of strong northerly winds, however, the incoming tide flows more rapidly over the flats, and waves with an amplitude approximating 0.3 m may be generated. Under these conditions the water over the flats becomes extremely turpid due to large amounts of surface sediment thrown into suspension. It is immediately after such periods of high winds that large amounts of material, including living clams, may be observed on the upper beach and in particular in the break in the beach profile, left there by the receding tide. It is felt that high winds, particularly those accompanying northeast storms, are chiefly responsible for the observed changes in location as well as the high degree of mortality of clam populations in this area.

REFERENCES

Eagleson, P. S., R. G. Dean, and L. A. Peralta. 1957. The mechanics of the motion of discrete spherical bottom sediment particles due to shoaling waves. Mass. Inst. of Tech., Dept. of Civil and Sanit. Eng., Hydrodynamics Lab. Tech. Report No. 26.

Ippen, A. T., and P. S. Eagleson. 1955. A study of sediment sorting by waves shoaling on a plane beach. Dept. of Army, Corps of Engineers, Beach Erosion Board Tech. Memo. No. 63.

Manohar, M. 1955. Mechanics of bottom sediment movement due to wave action. Dept. of Army, Corps of Engineers, Beach Erosion Board Tech. Memo. No. 75.

Miller, R. L., and J. M. Zeigler. 1958. A model relating dynamics and sediment pattern in equilibrium in the region of shoaling waves. breaker zone, and foreshore. Jour. of Geology, **66:** 417–441.

Rapson, A. M. 1952. The Toheroa, *Amphidisma ventricosum* Gray (Eulamellibranchiata), development and growth. Australian J. Mar. and Freshwater Res., **3:** 170–198.

Rector, R. L. 1954. Laboratory study of equilibrium profiles of beaches. Dept. of Army, Corps of Engineers, Beach Erosion Board Tech. Memo. No. 41.

Smith, O. R. 1955. Movements of small soft-shelled clams. U. S. Dept. of Int., Fish and Wildl. Serv., Spec. Sci. Rept. No. 159 (Fisheries).

Stephen, A. C. 1928. Notes on the biology of *Tellina tenuis* da Costa. J. Mar. Biol. Ass. U. K., **15:** 683–702.

———. 1929. Studies on the Scottish marine fauna: the fauna of the sandy and muddy areas of the tidal zone. Trans. Roy. Soc. Edinburgh, **56:** 291–306.

Stephenson, T. A., and A. Stephenson. 1949. The universal features of zonation between tide-marks on rocky coasts. J. Ecol., **37:** 289–305.

Turner, H. J. 1951. Fourth Report on Investigations of the Shellfisheries of Massachusetts. Div. Mar. Fish., Dept. of Conserv., Commonwealth of Mass., Boston.

Weymouth, F. W. 1923. The life-history and growth of the Pismo clam (*Tivela stultorum*). Calif. Fish. Bull. No. 7.

GEOGRAPHICAL ZONATION OF MARINE LITTORAL TYPES

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The tendency in recent years to generalize upon the extensive information about the fauna and flora of the littoral zone has resulted in such summary papers as those of Stephenson and Stephenson (1949), Dahl (1953), Pérès (1957a, b), Southward (1958), Doty (1957) and Hedgpeth (1957). Workers in the Soviet Union have also been active in this field, but much of their work has been published too recently to be available for the summaries mentioned above.

For several years the author has investigated the qualitative and quantitative distribution of the littoral fauna of the Okhotsk, Japanese and Black seas, as well as parts of the Azov, Berents, White, Bering, Yellow, East China seas (see references). This material, although not yet fully processed, permits us (with due consideration of the literature) to suggest some generalizations based on the postulates of Zenkevich (1949) on the biological structure of the ocean.

Latitudinal zonation of the biological phenomena on a global scale is primarily caused by the temperature factor in *sensu lato*. However, this climatic zonation is often disturbed by other factors, the most important being that of moistening affecting the amphibiotic littoral zone. The factor of moistening is determined by tidal range, or, more exactly, by the interaction of tidal range, surf conditions, and seasonal and sporadic changes of sea level. These conditions may greatly modify the general course of latitudinal zonation, increasing or reducing some geographical tendencies, as well as giving some specific features to the littoral zones of any type.

The interaction between tidal ranges, surf conditions, changes of sea level and some biotic factors to form the littoral or amphibiotic zone is shown schematically in Figure 1. If the tidal range is large, the unfavorable influence of seasonal and sporadic changes of sea level upon littoral life is smoothed down and weakened. Exposure to surf provokes some local changes in littoral populations, sometimes widening the habitat levels to a considerable degree, especially the upper ones. But the surf itself does not essentially change the moistening conditions of the littoral zone.

If the tidal range is small, the greater part of the littoral zone in exposed places is washed by the surf almost continuously. Here the surf action overlaps tidal action and the moistening conditions are quite different. On the other hand, even the presence of small tides cancels the unfavorable action of seasonal and sporadic changes of sea level on sheltered coasts, and on exposed coasts during calms.

In the absence of tides the seasonal and sporadic fluctuations of sea level hinder the development of plants and sessile animals in the littoral zone. They can develop only on shores permanently washed by surf action. The vagile inhabitants of the littoral may counteract the unfavorable changes of sea level by migration, while the sessile forms may survive only by a high resistance to prolonged exposure to air.

The systematic composition of the population of any coast is determined primarily by temperature, whereas such bionomic features as vertical zonation, distribution of organisms and communities, quantitative indices and relations are also largely influenced by moisture conditions. These conditions depend upon the tidal range. While most of the ecological environmental factors act locally, the factor of tidal range has a considerable regional extent, and moreover is subject to quasilatitudinal changes (Table 1). Small tides, or the absence of tides, are an essential feature of the great part of the Polar basin, as well as of many parts of the south-boreal, subtropical and tropical zones, and of the shores of Antarctica in the southern hemisphere (except Graham land).

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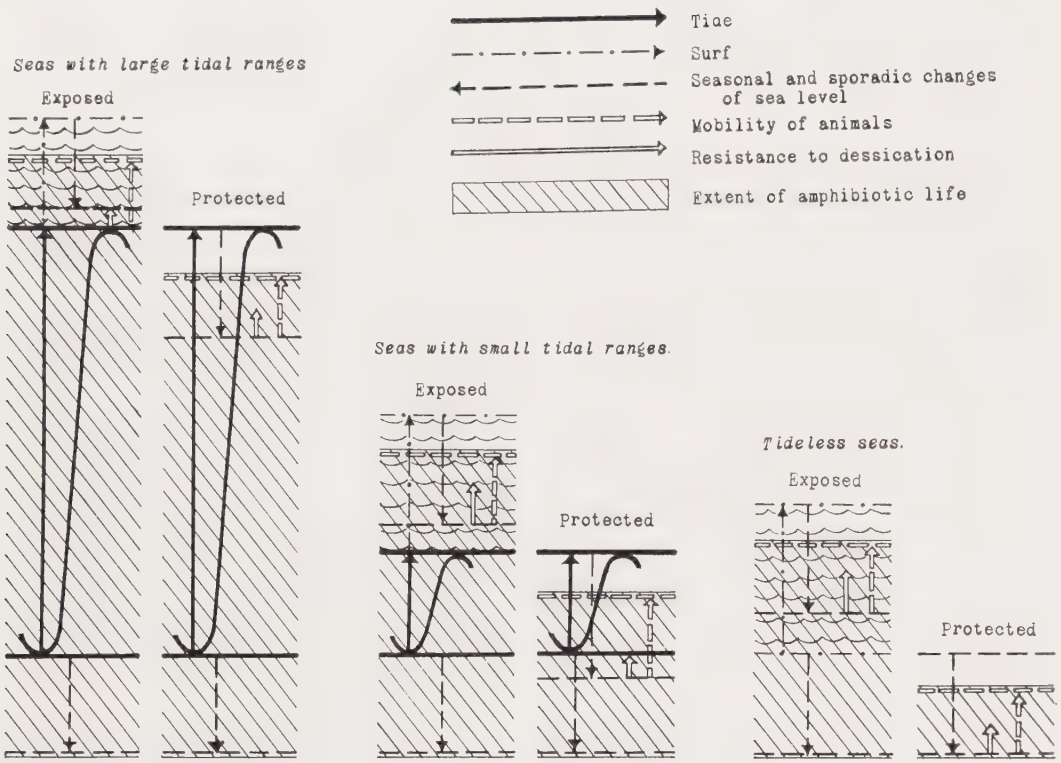

FIG. 1. Interaction of the factors forming the littoral as the zone of amphibiotic life.

Practically the whole north-boreal zone is characterized by a rather large tidal range.

We distinguish the different littoral types first, by their latitudinal-climatic characteristics, and secondly, by the dominant moisture conditions, which are expressed generally by the tidal ranges. Some particular types discussed here are based chiefly on our own material, with some comparative information from published sources.

It is generally accepted that the *high Arctic* littoral is barren of life. However, it seems that some kinds of littoral life do exist, although ephemeral in occurrence or as seasonal migrations into the sublittoral. Littoral life of this type requires further detailed investigations.

The subarctic littoral, as far as we can judge now, possesses neither biogeographical, nor, presumably, bionomic self-sufficiency. It shows only various degrees of depression, falling off and sinking into the sublittoral of the most eurybiontic boreal species. All authors (except Madsen 1936, 1940), who have investigated the subarctic littoral, considered this aspect only, paying no attention to possible specific manifestations of the littoral life mentioned above.

The inhabitants of the boreal littoral (and of the littoral of warmer latitudinal zones as well) demonstrate a clear tendency to an extremely eurybiontic mode of life, especially to eurythermy, which is determined by the peculiarities of amphibiotic life subject to abrupt changes of aquatic and aerial conditions, especially those of temperature. As a rule these littoral populations have an extraordinarily wide latitudinal distribution. We fully agree with the more detailed biogeographical divisions made by Andriashev (1939), Shschapova (1948) and others, but with respect to the littoral zone fewer divisions can be recognized. Thus, the littoral population of the boreal zone of the northwestern coast of the Pacific investigated by us (and, apparently, of the three other oceanic coasts) should be divided

TABLE 1. *Distribution of littoral types in the northern hemisphere*

Latitudinal zones	Large tides	Small tides	Practically no tides
Arctic	Hudson Bay Baffin Sea	Represented circumpolarly	—
North-boreal	All four north hemisphere coasts of the oceans	—	Baltic Sea
South-boreal	Virginia, Bay of Biscay, W. coast Pacific coast Pyrennean penins., California	Japanese Sea (Primorye; S.W. Sakhalin)	Black Sea, Sea of Azov Caspian Sea
Subtropical	of Japan, Yellow Sea, Morocco	Mediterranean Sea, Gulf of Mexico	—
Tropical	Represented circumtropically	Caribbean Sea, Red Sea, some islands of Oceania	—

only into two biogeographical subareas: north-boreal and south-boreal.

The littoral zone of almost the whole Okhotsk Sea, the greater part of the Bering sea, the eastern coast of Kamchatka, the greater part of the Kurile Islands and of the northern part of the Tartar Strait, is very similar both biogeographically and bionomically. We refer the littoral fauna and flora of all these coasts to one single type: the *north-boreal littoral type with appreciably large tides.*

This is a "classic" littoral with a vertical zonation according to Vaillant's (1891) scheme. Relatively uniform populations of rather large perennial plants and of animals with life-spans of several years are dominant. An intensive development of fucoid growths on hard surfaces and a rather monotonous fauna is specific for this type of littoral. The fauna of unconsolidated sediments is more varied. The biomass indices are high, but the rate of production is rather low. Seasonal changes of the flora and the fauna are relatively slight, especially as contrasted to the very abrupt climatic changes. By comparing the north-boreal littoral of the Far East with that of the Murman coast of the Barents Sea, we notice an amazing similarity and often even an identity in the systematic composition of species and parallel communities (Tables 2 and 3), as well as a close resemblance in zonation and in qualitative indices (Table 4). These significant similarities emphasize that the differences in types of tides play no important part in the littoral bionomy. In fact, the Murman littoral is formed by regular semidiurnal tides and the Far-Eastern littoral by irregular semidiurnal and diurnal tides. These differences provoke merely a certain shifting of some critical levels in the vertical distribution of the organisms. The increase of the range from 1.5–2.5 m in the Tartar Strait, or from 3–5 m on the Shantar Islands and Ayan, to 10–13 m in the Shelikhov Bay, are of still less bionomic importance. In these cases the limits of vertical distribution are stretched or compressed like the bellows of an accordion, since the moistening conditions remain essentially the same.

According to published information, the Murman littoral has characteristics in common with a great part of the northwestern Atlantic.

Ecological and systematic comparison of species (Tables 1 and 2) and of communities may be extended on one hand to the Atlantic coast, and on the other, to the Pacific coast of North America. Both of the opposite coasts of corresponding oceans ought to demonstrate a large similarity in the composition of the common species and in zonation. According to the literature the littoral of the coasts from the Gulf of St. Lawrence to Virginia, and from Alaska

TABLE 2. *Comparison between the Common species of the littoral fauna of Barents and Okhotsk seas*

Barents sea	Okhotsk sea
Rocks. Seaweed growths	
Littorina saxatilis rudis Littorina obtusata	Littorina sitchana subtenebrosa
Littorina littorea	Littorina squalida
Acmaea testudinalis	Acmaea testudinalis
Nucella lapillus	Thais lima
Lucuna vincta	Lacuna vincta
Skeneopsis planorbis	
Margarites helicinus	Margarites helicinus
Balanus balanoides	Balanus balanoides Chthamalus dalli
Jaera marina	
Hyale prevostii	Hyale ochotensis
	Nototropis collingii
Gammarellus homari	Gammarellus homari
Calliopius laeviusculus	Calliopius laeviusculus
Amphithoe rubricata	Amphithoe rubricata Amphithoe nov. sp.
Haliclystus octoradiatus	Haliclystus steinegeri
Stony ground	
	Nereis vexillosa
Nereis zonata	Nereis zonata
Harmothoe imbricata	Harmothoe imbricata
Tonicella rubra	Tonicella rubra
Asterias rubens	Evasterias retifera Leptasterias ochotensis
Henricia sanguinolenta	Henricia sp.
	Harenactis sp. (?)
Gammarus ex gr. locusta Gammarus ex gr. marinus	Gammarus locustoides Echinogammarus spasskii Anisogammarus subcarinatus Marinogammarus 2 nov. sp.
	Pontogeneia (2 spp.)
Idothea granulosa Idothea baltica	Idothea ochotensis
Pagurus pubescens	Pagurus middendorffii

probably to Oregon, have the characteristics of the north-boreal type with large tides. Unfortunately the almost complete absence of any qualitative data for these coasts makes a comprehensive comparison impossible.

Our material from the south-boreal zone comes only from seas with small tides or without tides. This does not permit us to trace the course of latitudinal changes not subject to the modifying influence of small tides on the littoral.

The south-boreal littoral type with small tides is well marked in the Japanese Sea, on its continental coast, and in some impoverished condition on southwestern Sakhalin as well. Here the fauna of unconsolidated sediments is poor, both qualitatively and quantitatively. The peculiar rocky littoral is divided into two horizons. The upper hori-

TABLE 3. *Comparison between the Common species of the littoral fauna of Barents and Okhotsk sea () indicate only below ELTM*

Silty—sand beaches	
Barents sea	Okhotsk sea
Macoma baltica	Macoma baltica
(Liocyma fluctuosa)	Liocyma fluctuosa
Mya arenaria	Mya arenaria
Mya truncata	Myra truncata
Cardium edule	Cardium californiense
Echiurus echiurus	Echiurus echiurus
Priapulus caudatus	Priapulus caudatus
Halicryptus spinulosus	Halicryptus spinulosus
(Golfongia margaritacea)	Golfingia pavlenkoi
(Mesidothea enthomon)	Mesidothea enthomon orientalis
Corophium crassicorne	Corophium steinegeri
	Chiridota tauiensis
Arenicola marina	Arenicola marina schantarica Arenicola claparedii
Amphicora fabricii	
	Chone teres
Scoloplos armiger	Scoloplos armiger
Pygospio elegans	Pygospio elegans
Spio filicornis	Spio filicornis
Eteone longa	Eteone longa
Nephthys longosetosa	Nephthys longosetosa
Nephthys coeca	Nephthys coeca
Pectinaria koreni	Pectinaria granulata
Travisia forbesii	Travisia forbesii
	Glycinde armigera
Capitella capitata	Capitella capitata

TABLE 4. *Mean biomass (in g/m²) of some species in terms of their populations. (Preliminary data)*

Seas	Okhotsk		Barents	
Populations / Coast	East-Sakhalien	North coast of Okhotsk sea	West Murman coast (after L. Zenkevich)	East-Murman coast
Littorina of upper littoral	271	109	223	252
Balanus balanoides (+*Chthamalus* in the Far East)	1112	1915	1175	1200
Mytilus edulis	4963	4878	4806	4163
Macoma baltica	233	306	204	104

zon is very similar to Vaillant's first horizon of the north-boreal littoral. The lower horizon of this littoral type is filled by the dense mosaical growths of Rhodophyceae with various and extremely abundant fauna (10,000–200,000 individuals/m²). Small Crustacea, especially Amphipoda, prevail in this fauna. Here the biomass is not so great (1,700–2,500 g/m² for the plants, 50–150 g/m² for the animals), but the rate of production is very high. Seasonal changes are very sharp. These changes are due not only to the climatic cycle, but also to the seasonal changes of the sea level which cannot be overlapped by the small tidal range.

Most of the above features are also characteristic of the Mediterranean Sea with its small tides. However, its *subtropical* aspect is manifested, particularly in the prevalence of the hard calcareous Rhodophyceae (for instance the famous "trottoires" of *Lithophyllum tortuosum*) over uncalcified algae.

TABLE 5. *Parallel communities of the lower littoral of small tide seas*
Fauna of Rhodophyceae turf

Sea of Japan	Gulf of Mexico (after Hedgpeth 1950)
	Carinogammarus mucronatus
Caprella acutifrons	Caprella acutifrons
Jassa pulchella	Jassa marmorata
Ischyrocerus rhodomelae Ischyrocerus cristatus	Ischyrocerus sp.
Amphithoe japonica Amphithoe rubricata	Amphithoe valida
Melita koreana	Melita nitida
Hyale bassargini	Myale havaiensis
Parhyale zibellina	
Allorchestes malleolus	
Allorchestes plumicornis	
Leptamphopus littoralis	
Pontogeneia rostrata	

In algal turfs the small Mollusca prevail over the Amphipoda. The communities of unconsolidated sediments, according to Giordani Soika (1949), are similar to the communities of the Sea of Japan both in qualitative composition and in quantitative relations. The small tide littoral of the Gulf of Mexico described by Hedgpeth (1953), shows some similarities to this south-boreal type. In Table 5 a specific composition of all the common Amphipoda inhabiting the mosaical turf of red algae of the lower littoral of the Sea of Japan and the Gulf of Mexico is given.

In the south-boreal tideless seas (the Black Sea, the Sea of Azov, the Caspian Sea, and in the north-boreal zone the Baltic Sea as well) a surfy littoral of a peculiar type is developed, which we term *pseudolittoral.* Specific and rather productive communities of the unconsolidated sediments consist of 1 or 2 to 6 constant animal components. Here the biomass is several times greater than that of the Japanese or the Mediterranean seas.

In contrast, the population of the rocky littoral, especially the algal flora, is sharply depressed. Seasonal and sporadic changes of sea level hinder development of the littoral population on rocks. Except for points where surf is extreme, the population is represented either by the mobile organisms and their epiphytic algae, or by ephemeral species. The peculiarities in the composition of the pseudolittoral population of the southern seas of USSR are due primarily to salinity conditions. In other parts of the globe this littoral type is absent.

Suptropical and tropical littoral types with rather large tides in the Chinese seas: the subtropical littoral of the Yellow Sea (and the greater part of the East-Chinese Sea as well) is populated by a greatly impoverished tropical fauna and therefore should not be attributed to an independent biogeographical area or subarea, as was already noted by Guryanova (1958). But bionomically it is distinct enough from the tropical and the south-boreal littoral and may be distinguished as a self-sufficient type.

This littoral type is characterized by an increasing variety of almost all faunal groups (except Amphipoda and Peracarida in general), but there is at the same time a qualitative and quantitative reduction of the flora. The biomass indices of the algae seldom exceed 1 kg/m^2.

Organisms with massive calcareous skeletons (*Ostrea* spp., Cirripedia, Thoracica) prevail in the epifauna and cryptofauna of the rocks. Quantitative indices for their communities are comparable with the indices for the rocky communities of the north-boreal littoral (those of *Mytilus edulis* and of *Balanus balanoides*). In contrast, the

qualitatively rich fauna of the unconsolidated sediments is quantitatively impoverished. Thus the biomass fluctuates usually within the limits of 50 g/m^2, seldom 100 g/m^2 as contrasted to 200 g/m^2 and often more in the Okhotsk Sea. Comparing our data with that of Guryanova (1958) for the Yellow Sea littoral and of Dun Yu-mao (1956) for the East-Chinese Sea, obtained in both cases in summer, some seasonal changes could be established. Thus it was found that some common crab species disappear from the upper littoral during the autumn and winter. These seasonal changes are insignificant as compared with the abrupt changes observed in the south-boreal littoral with small tides.

The *tropical littoral* is characterized by two communities of the highest order: mangrove swamps and coral reefs. In other respects the trends already manifest in the subtropical littoral are here more distinctly developed by progressive reduction of hard bottom algal flora and by qualitative enrichment of fauna together with quantitative impoverishment. The impoverishment is especially expressed in unconsolidated sediments. Usually the biomass fails to exceed 10 g/m^2 and frequently is in fractions of a gram. The fauna of the silt and silty-sand bottoms of the mangrove swamps is some what richer, though qualitatively very similar to that of sediments with similar composition without mangroves. The epifauna of the mangrove stems is almost devoid of indigenous species and consists of forms from rocky stony bottoms but in lesser numbers and variety. Seasonal changes are apparently very feebly expressed in the tropical littoral of the South China Sea. This is not a general feature of the whole tropical littoral; there are regions with very strong and even catastrophic seasonal changes associated with monsoons, as on the Malabar coast (Seshappa 1953).

There are frequent statements in the literature about the gradual lowering of the upper boundaries of the whole littoral population into the lower littoral and even into the sublittoral zone, as one moves from the temperate latitudes to the equator. But our data from the Chinese seas indicate that this statement is correct only for the algal flora. With respect to the fauna, similar lowering is restricted only to single biotopes (for instance, the coral sand beaches). In the majority of biotopes the littoral zone is inhabited throughout. Here the "azoic horizons" due to the action of the local conditions are not more widely distributed than they are in the north- or south-boreal littoral zones. The information from the available literature suggests that these regularities are repeated inversely in the southern hemisphere from the equator to the Antarctic (biological bipolarity); future study may provide interesting material bearing on the biological structure of the ocean.

Some distinctions are already apparent. First, there is much less land surface in the cold-temperate zone of the southern hemisphere (south-notal zone in our terminology) than in the northern hemisphere, and therefore as stated by Southward (1959) the cold-temperate littoral fauna is but slightly expressed there. Second, the small-tide-littoral, which disturbs the smooth course of climatic zonation in the northern hemisphere, is almost entirely absent in the southern hemisphere. Hence the changes of latitudinally climatic zonation in the littoral region should be more pronounced on the coasts of South America, which are still insufficiently investigated.

REFERENCES

Andriashev, A. P. 1939. The fishes of the Bering Sea and neighbouring waters, its origin and zoogeography. Leningrad. 185 pp., 16 figs. (In Russian, with English summary as titled, pp. 181–185.)

Dahl, Erik. 1953. Some aspects of the ecology and zonation of the fauna of sandy beaches. Oikos, **4**(1): 1027, 8 figs.

Doty, Maxwell S. 1957. Rocky intertidal surfaces, *in* Treatise on Marine Ecology and Paleoecology, Geol. Soc. America, Mem. **67**, Vol. 1: 535–585, 18 figs., 1 pl.

Dun Yu-Mao. 1956. The ecological types of animals of the Putuoshang Island, Chowsang Archipelago. Bull. Pedagogic Univ. Chekiang, **12**: 245–251, 14 figs. (In Chinese.)

Giordani Soika, A. 1955. Ricerche sull' ecologia e sul popolmento della zona intercotidal dell spiagge di sabbia fina. Boll. Mus. Civ. Stor. Nat. Venezia, **7**: 5–151, 67 figs. (In Italian.)

GORYANOVA, EU. F. 1958. Investigations of the Yellow Sea. Reports of the Soviet-Chinese Expedition. Vestnikh Akad. Nauk. USSR, **2**: 104–105. (In Russian.)

———. 1959a. Marine zoological expedition to Hainan Island. *Ibid.*, **3**: 89–92. (In Russian.)

———. 1959b. Investigations of the drying zones of Chinese seas. Izvest. Akad. Nauk, SSSR, ser. biol., **5**: 741–758, 5 figs. (In Russian.)

———, LIU, J.-Y., SKARLATO, O. A. USHAKOV, P. V., WU BAO-LIN, AND TSI CHUNG-YEN. 1958. Characteristics of the littoral of the Yellow Sea. Bull. Biol. Marin. Inst. Acad. Sinica, **1**: 22–41, 3 figs. (In Russian.)

HEDGPETH, JOEL W. 1950. Appendix, *in* Whitten, Rosene and Hedgpeth, the Invertebrate fauna of Texas Coast jetties. Publ. Inst. Mar. Sci. Texas, **1**: 72–85.

———. 1952. Intertidal zonation and related matters, *in* Ricketts and Calvin, *Between Pacific Tides*: 375–401, Stanford University Press.

———. 1953. An introduction to the zoogeography of the northwestern Gulf of Mexico with reference to the invertebrate fauna. Publ. Inst. Mar. Sci. Texas, **3**: 109–224, 45 figs.

———. 1957. Sandy beaches, *in* Treatise on Marine Ecology and Paleoecology, Geol. Soc. America, Mem. **67**:, Vol. 1: 587–608, 11 figs., 1 pl.

MADSEN, H. 1936. Investigations on the shore fauna at east Greenland, with a survey of the shores of other Arctic regions. Medd. Grønl., **100**(8): 1–79.

———. 1940. A study of the littoral fauna of northwest Greenland. *Ibid.*, **124**(3): 1–24.

MOKYEVSKY, O. B. 1949a. Fauna of unconsolidated sediments of the littoral of the Crimea. Trudy Inst. Okeanol., **4**: 124–159, 8 figs. (In Russian.)

———. 1949b. Fresh-water littoral of Amur liman. Dokl. Akad Nauk SSSR, **66**(6): 1187–1190. (In Russian.)

———. 1953. Littoral fauna of the Sea of Okhotsk. Trudy Inst. Okeanol., **7**: 167–197, 2 figs. (In Russian.)

———. 1956. Considerations of the littoral fauna of the continental shore of the Sea of Japan. Trudy probl. temat. sov Zool. Inst. AN SSSR, **6**: 116–121 (Russian).

———. 1959. Littoral fauna of the northwestern shore of the Sea of Japan. Trudy Inst. Okeanol., **34**: 242–328, 9 figs.

PÉRÈS, J. M. 1957a. Le problème de l'étagement des formations benthiques. Réc. trav. Stat. mar. d'Endoume, **21**, (Bull. 12): 3–21. (In French.)

———. 1957b. Essai de classement du communités benthiques marines du globe. *Ibid.*, **22**: 23–54. (In French.)

SESHAPPA, G. 1953. Observations on the physical and biological features of the inshore sea bottom along the Malabar coast. Proc. Nat. Inst. Sci. India, **19**(2): 257–279.

SHSCHAPOVA, T. F. 1948. Geographic distribution of representatives of the order Laminariales in the northern Pacific Ocean. Trudy Inst. Okeanol., **2**: 59–138, 5 figs. (In Russian.)

———, MOKYEVSKY, O. B., AND PASTERNAK, F. A. 1957a. Flora and fauna of coastal zones of Putjatin Island (Japan Sea). Part I. Qualitative composition. *Ibid.*, **23**: 67–101, 1 fig. (In Russian: English title as given in table of contents.)

———. 1957b. Flora and fauna of the littoral of Western Sakhalin. (Preliminary report.) *Idem*: 102–111, 1 fig. (*Idem.*)

SOUTHWARD, A. J. 1958. The zonation of plants an animals on rocky sea shores. Biol. Rev., **33**: 137–177.

STEPHENSON, T. A. AND ANNE. 1949. The universal features of zonation between tide-marks on rocky coasts. J. Ecol., **37**: 289–305.

VAILLANT, L. 1891. Nouvelles études sur les zones littorales. Ann. sci. nat., (Ser. 7: zool.) **12**: 39–50. (In French.)

ZENKEVICH, L. A. 1949. La structure biologique de l'ocean, C. R. XIII^{ème} Congrès int. Zool., pp. 522–529, 7 figs. (In French.)

ON INTERNAL WAVES IN LAKES

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ABSTRACT

The dominant feature of short period thermal unrest in a stratified lake is the periodic nature of the temperature variation. When such variations at a point occur within a few minutes, periodic vertical motions of the water are implied, for other heat transfer mechanisms within the lake are too slow. These periodic motions, internal waves, may be studied at a given depth by simultaneous continuous temperature measurement at three closely spaced stations. From these observations and the mean vertical gradient the amplitude, direction, period, and speed of the internal waves have been determined.

Long, standing internal waves have been described often in the literature as seen through temperature observations. They may also be studied by means of their "surface reflection."

A remote reading thermometer with little time lag suspended in Lake Mendota will rarely indicate a steady value. This fact, in itself, would not be surprising if the fluctuations were nearly random and there were some evidence of turbulent motion, but even with a completely smooth surface and sufficient stratification to suppress turbulence, the temperature unrest continues. The obviously periodic nature of the record obtained on many occasions is also very striking. Whether near the surface, within the epilimnion, at the thermocline or below, these temperature oscillations of a few minutes duration and up to 2°C amplitude are more characteristic than a state of quietude.

That the temperature unrest is associated with motions of the water is hardly to be questioned. No other possible cause comes to mind. Furthermore, periodic motions of the same general frequency have been observed: both oscillation of the horizontal current direction (Lathbury, *et al.* 1959) and of the relative vorticity.

The periodic nature of the phenomenon, its occurrence at all levels in the lake, and its apparent lack of relationship to the state of the sea at the surface suggest that *internal waves* were being observed.

THE NATURE OF INTERNAL WAVES

Internal waves are an inherent characteristic of any disturbed *stratified* medium, *i.e.*, a medium in which buoyant forces tend to restore a disturbed parcel to its original position. They should be found, therefore, at the thermocline, in the hypolimnion, in the epilimnion when it is stratified, and in the atmosphere as well (Bryson 1948). Indeed, surface waves are a special case of internal waves where the medium as well as the density changes in the vertical and the density of the upper medium is negligible compared to that of the lower.

The theory of internal waves was first treated by Stokes (1847). Excellent discussions of the theory are to be found in Sverdrup (1946) and Proudman (1953). These are summarized below for certain special cases.

A quadratic expression for the phase velocity may be derived with the roots

$$c_1^2 = \frac{gL}{2\pi}; \quad c_2^2 = \frac{gL}{2\pi} \frac{\rho - \rho'}{\rho \coth kh' + \rho'} \quad (1)$$

where c_1 = phase velocity of surface waves
c_2 = phase velocity of internal waves
g = gravity
L = wave length
ρ = density of lower layer
ρ' = density of upper layer
h = depth of lower layer
h' = depth of upper layer
$k = \frac{2\pi}{L}$ = wave number

This expression applies approximately for short waves, that is $L << (h + h')$.

For long internal waves, the corresponding roots are

$$c_1^2 = g\,(h + h'); \quad c_2^2 = \frac{g\,h\,h'}{h + h'}\,\frac{\rho - \rho'}{\rho} \qquad (2)$$

A further approximation for the case where h is large compared to h′ gives

$$c_1^2 = \frac{gL}{2\pi}; \quad c_2^2 = gh'\,\frac{\rho - \rho'}{\rho} \qquad (3)$$

The maximum amplitude η_{max} is at the boundary surface. At the water–air interface, the amplitude η_o is reduced to

$$\eta_o = \frac{-\eta_{max}\,(\rho - \rho')}{\rho} \qquad (4)$$

the negative sign indicating a reversal of phase. There is then a level between these two with no vertical displacement, that is a nodal surface.

Long internal waves in a lake are represented primarily by the standing internal waves or "temperature seiche" well known to limnologists (Wedderburn and Williams 1911). The authors are not aware of any literature dealing with short internal waves in lakes. Furthermore, there have been relatively few observations made on short internal waves in the sea.

It is important to note at this point that the energy per unit area of an internal wave is given approximately by

$$E = \tfrac{1}{2}\,(\rho - \rho')\,g\,\eta_{max}^2 \qquad (5)$$

while that of a surface wave is given by

$$E = \tfrac{1}{2}\,\rho'\,g\,\eta_o^2 \qquad (6)$$

This means that waves on a weak thermocline may be of very large amplitude and still not represent as much energy as rather small surface waves (Table 1).

TABLE 1. *Approximate wave height of internal waves at the thermocline with the same energy as surface waves of given heights for given temperatures of epi- and hypolimnion*

Surface Wave Height (m)	Epilimnion / Hypolimnion	6 / 4	10 / 5	15 / 7	20 / 10	25°C / 10°C
0.01		18	0.62	0.35	0.26	0.19
0.05		90	3	2	1.3	0.95
0.10		180	6	3.5	2.5	2
0.20		—	12	7	5	4

FURTHER THEORETICAL DEVELOPMENTS

In the previous paragraphs certain theoretical conclusions with regard to a surface of discontinuity in density were described. Fjeldstad (1933) discussed the case for continuous variation in density. His conclusion was that an infinite number of internal waves could be simultaneously present each with a different number of nodes between surface and bottom, and each with its own phase angle.

Most oceanic verification of the importance of internal waves has been with reference to long waves, especially of tidal period (Helland-Hansen and Nansen 1909, 1930; Seiwell 1937; Ekman and Helland-Hansen 1931; Defant 1932; Lek 1938). Considerable material on internal waves at sea, especially with regard to their stability, was summarized by Defant (1949).

On the other hand, observations of short internal waves at sea are rare (Ufford 1947 a, b, c; Neumann 1949) and are apparently nonexistent for lakes.

In a lake, the thermocline sometimes resembles a true surface of discontinuity, sometimes the continuous density variation of Fjeldstad (1933), but more often is a finite layer of density transition between rather uniform epilimnion and hypolimnion. This case has been treated by Groen (1948), whose results are summarized here:

Groen shows that if the specific volume S is given by the distribution

$$S\,(z) = S_o + \tfrac{1}{2}\,\Delta S\,\mathrm{tgh}\,(2z/b) \qquad (7)$$

and if the fluid is sufficiently deep on both sides of the transition layer then the relation between the wave length ($L = 2\pi\lambda$) and period ($T = 2\pi\tau$) is given by

$$\frac{g\,\Delta S}{b\,S_o\,\tau^2} = n\,(n+1)\left(\frac{2\lambda}{b}\right)^2 + (2n+1)\,\frac{2\lambda}{b} + 1 \qquad (8)$$

where $n = 0, 1, 2, 3 \ldots$, the latter representing the mode of oscillation (number of nodes in the vertical profile of oscillation). For $L \to 0$ the period has a minimum value given by

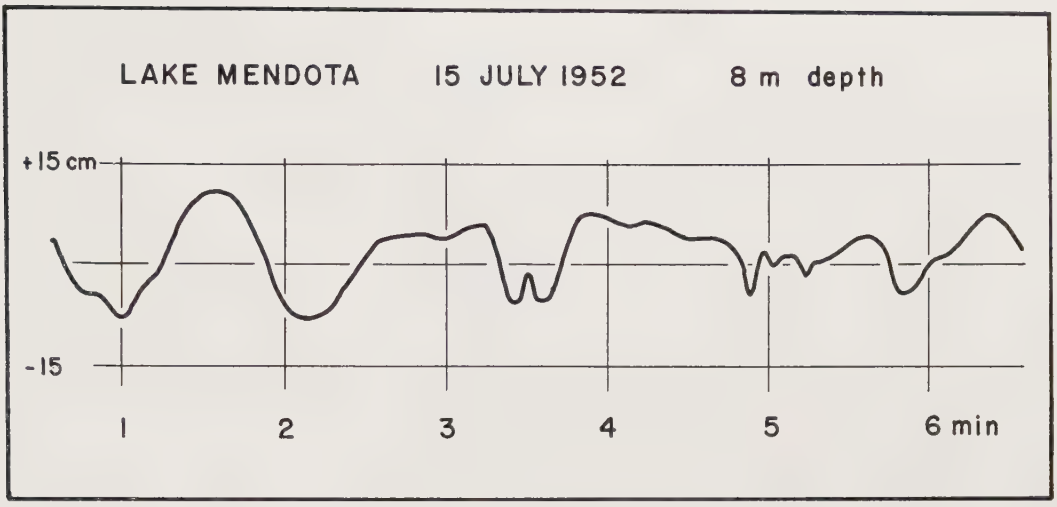

FIG. 1. Variation in the depth of an isotherm about its mean depth of 8 m in University Bay, Lake Mendota, 15 July 1952. It is evident that the variation is roughly periodic, but consisting of several components. Based on a continuous record.

$$T_{min} = \frac{2\pi}{\sqrt{g\,(S^{-1}\,ds/dz)_{max}}} \qquad (9)$$

which is independent of n.

Groen states that: "The existence of this lower limit of the period of internal waves appears to be a general feature, not restricted to the special type of density distribution assumed here."

A somewhat different approach has been taken by Neumann (1949) to explain periodic "thermal unrest" observed in the Baltic Sea. He postulates counter-rotating convection cells with horizontal axes (probably the same as Langmuir's horizontal vortices) which oscillate, thus giving rise to short standing internal waves. His mathematical development yields an expression for the period similar to that of Groen except for a dependence on dimension due to the standing nature of the waves. All the temperature records given by Neumann are for a single point and do not, therefore, eliminate the possibility that the waves are not of the standing type.

FIELD STUDIES

Evidence for short internal waves in Lake Mendota is of several sorts. The first indications were obtained by serial observations at a fixed depth and fixed location.

Figure 1 shows the results of a temperature recording at 8-m depth. Temperature has been converted to vertical motion from a temperature-depth curve. There is periodicity of about one minute and a vertical displacement of 15 to 25 cm. The record is clearly not one of a single sinusoidal variation. Of the many such observations made during 1949–1953, simple patterns have been rare. A record of one of these is given in Figure 2. In general, complex patterns as in Figure 1 are far more common.

Late in 1952 and during the summer of 1953, a tripartite method for measuring internal waves was adopted (Ufford 1947b). By this method the temperature fluctuations at three fixed points in a horizontal plane are recorded. Fluctuations associated with progressive internal waves will appear at each of the three points at different times. If the fixed points are appropriately spaced, the phase differences may be analyzed to obtain not only the wave periods but also the direction of progress and speed of the waves and hence their length.

The rig used to obtain most of the measurements reported here is shown diagrammatically in Figure 3. The temperature elements were 10-junction thermopiles. One set of couples was exposed directly to the water while the reference junctions were

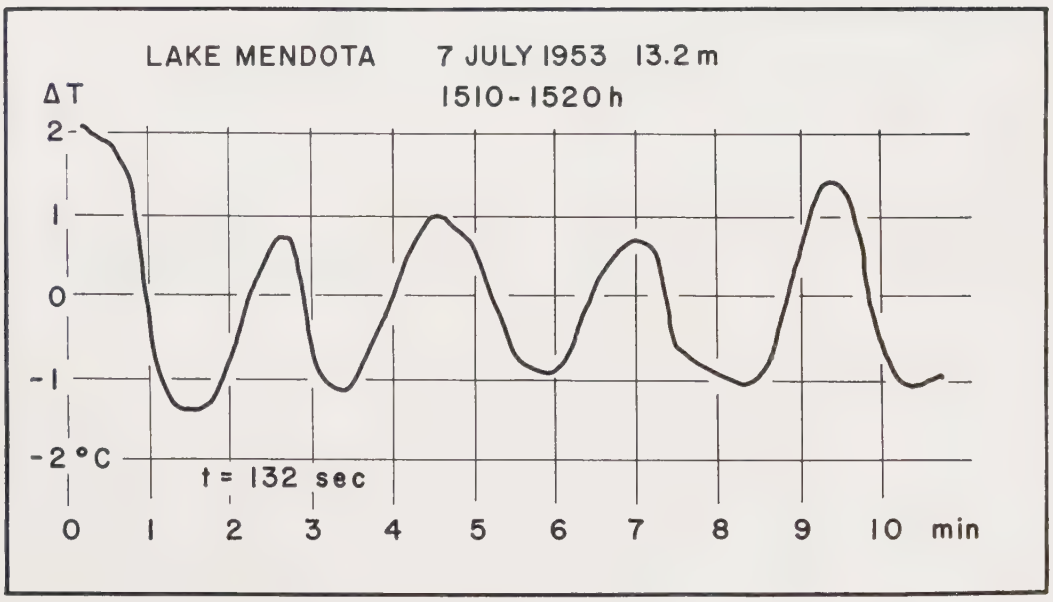

FIG. 2. A record of temperature variation at 13.2 m on 7 July 1953. This illustrates the simple periodic variation sometimes observed in Lake Mendota.

imbedded in beeswax so increasing their time constant to about half an hour which was much larger than the expected period of the waves. All thermopiles used were carefully checked to be sure the time constant was the same in each case. Thus, the temperature anomaly due to the internal wave was measured as a departure from the mean temperature at the depth of measurement. Thermopiles were mounted on the vertices of a triangle with 528-cm sides (see Fig. 3). The entire rig was suspended from submerged buoys so that it could not tilt, rotate, nor move up and down.

The thermocouple outputs were amplified by a portable chopper-type amplifier (designed by Prof. V. E. Suomi and built by Mr. C. R. Stearns) and recorded on an Esterline-Angus 0–1 ma portable recorder.

The input was switched from one thermopile to the next each 4 to 5 sec. For purposes of analysis the records so obtained were re-plotted on a compressed time scale and an expanded temperature scale. All three thermopile records were plotted on the same time scale (Fig. 4). The phase lags among the three curves were determined by tracing one of the curves on a transparent overlay and shifting it along the time scale until it best matched each of the other two curves. This was repeated by tracing all three curves and comparing the results. Where poor agreement was found, analysis was not attempted.

As the speed of the waves may be regarded as essentially constant, the phase differences may be used to calculate the direction of progress of the wave, speed, and hence the wave length. This is done geometrically by using the diagram in Figure 3. The angle α is found by graphical solution of the equation

$$\frac{A'C}{B'C} = \frac{\cos \alpha}{\cos (60 - \alpha)}$$

Then, since the orientation of the frame is known, the direction of progress of the wave can be determined. The length of A′C divided by the time interval of A′C gives the speed of the wave, c, and the wave length, L, is obtained by the relationship ct = L, where t is the wave period.

LAKE MENDOTA

In June, 1953, a station for measuring progressive internal waves by the tripartite

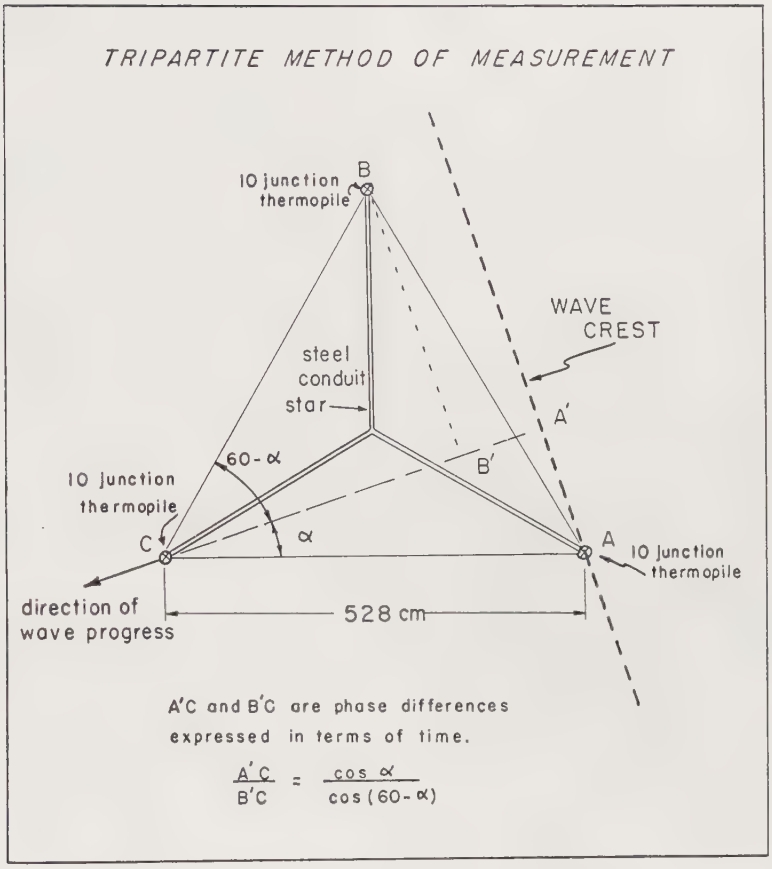

FIG. 3. Layout of the thermopile rig used to determine the direction and velocity of progressive internal waves. From the known orientation of the rig and the computed angle α, the direction of progress was derived. The velocity could then be determined from the time difference A'C.

method was established 2400 ft north of the University of Wisconsin Hydrobiological Laboratory in water 17 m deep. A total of 11 tripartite records and 6 single thermopile records were obtained at this station. Of these, 7 of the tripartite records could be analyzed for period, direction of travel, speed, and length, and 6 of the remaining records of both types were analyzed for period only. One other tripartite measurement was made on October 9, 1952, by use of three Whitney Electric Thermometers which were suspended from two securely anchored boats. Temperatures were read and recorded at 5-sec intervals. This measurement was made at a point about one-half mile south-southwest of Governor's Island.

Typical tripartite records are shown in Figures 4 and 5. The amplitudes are most conveniently expressed in terms of degrees Centigrade; however, they have been approximately converted to centimeters from the vertical temperature distribution measured sometime during the record interval. The results of all analyzed records are shown in Table 2. Most of the measurements are for the 10.7-m level which lay within the thermocline during the period July 9–16. None of the observed values for the period, t, are less than the theoretical minimum period, t_{min}, calculated by the theory of Groen.

Comparison of the directions of the wind and internal waves shows that, in general,

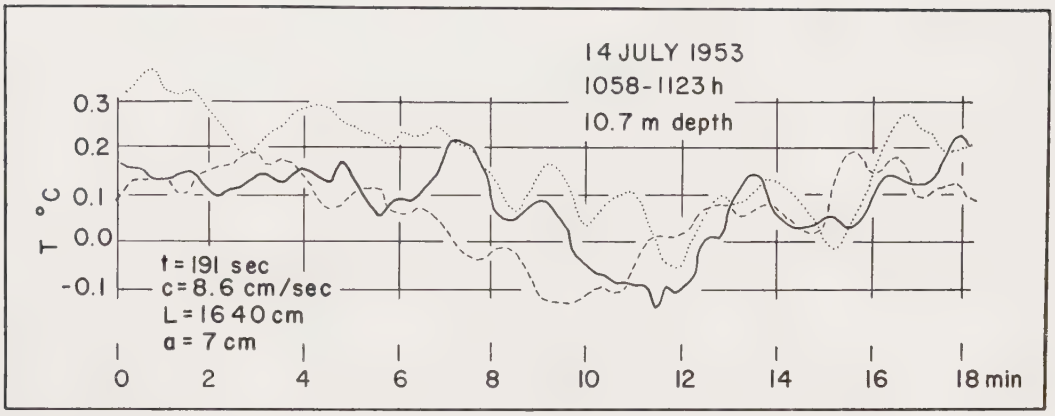

FIG. 4. Tripartite temperature variation record on 14 July 1953. The three records are from the corners of the rig shown in Figure 3.

the internal waves in the region of the thermocline move to the right of the wind. There seems to be no pronounced tendency for the waves to move upwind. Hence, the origin of these waves is not at the downwind end of the lake. This is surprising since the concentration of kinetic energy in the form of surface waves and turbulence is probably highest at the downwind end of the lake. Where and how progressive internal waves originate remains unknown.

TROUT LAKE

Trout Lake which is in Vilas County, Wisconsin, is composed of two basins connected by a narrow channel. This channel is approximately 425 ft wide and 720 ft long and has a maximum depth of slightly more than

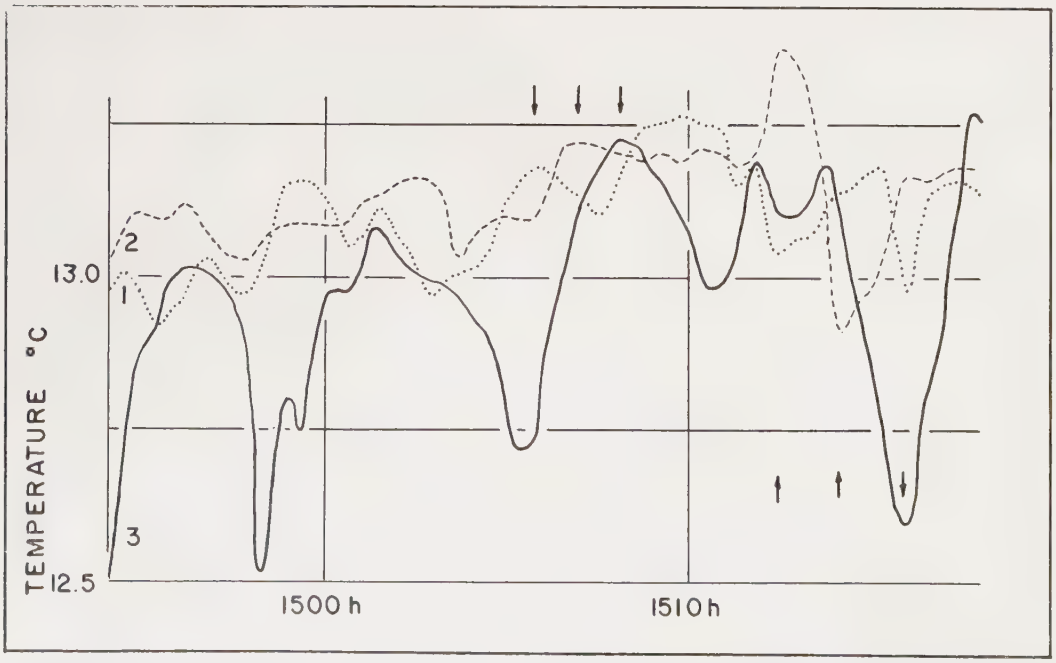

FIG. 5. Tripartite temperature variation record, using Whitney direct reading resistance thermometers, near Governor's Island, Lake Mendota, on 9 October 1952. Depth 16 m. Observations made every 5 sec.

TABLE 2. *Summary of Lake Mendota internal wave measurements*

Date	Time	Location	Depth(m)	Major Period, t (sec)	Length L (cm)	Speed c (cm/sec)	Amplitude, a (cm)	Direction of Progress°	Calculated Minimum Period by Groen's Theory (sec)
521009	1500–1520	Gov. Is.	16	330	1000	3	100	20	162
530629	1400	17 m sta.	8	187	1610	8.6	10	44	103
530707	1500–1530	"	13.1	132			10		57
520707	1558–1608	"	12	90			1		
530709	1400–1411	"	10.7	233	744	2.42	1	287	72
530709	1537–1550	"	10.7	270			1		
530709	1552–1601	"	10.7	180			0.5		56
530710	1527–1547	"	10.7	319	1746	5.4	5–10	205	82
530711	1124–1147	"	10.7	200	1540	7.7	8	190	98
530714	1058–1123	"	10.7	150	1105	6.7	7	303	87
530715	0955–1010	"	10.7	163	388	2.38	6	119	88
530715	1016–1044	"	10.7	474	3010	6.35	6	325	101
530716	0846–0902	"	10.7	109			10		83
530716	0902–0914	"	10.7	109			15		84

15 m. Several measurements of internal waves were made at the south end of the channel. Because of the configuration of the channel, it seemed reasonable to assume that if internal waves were present, they would probably move through the channel parallel to it. Therefore, a bipartite system with two thermopiles, one meter apart hori-

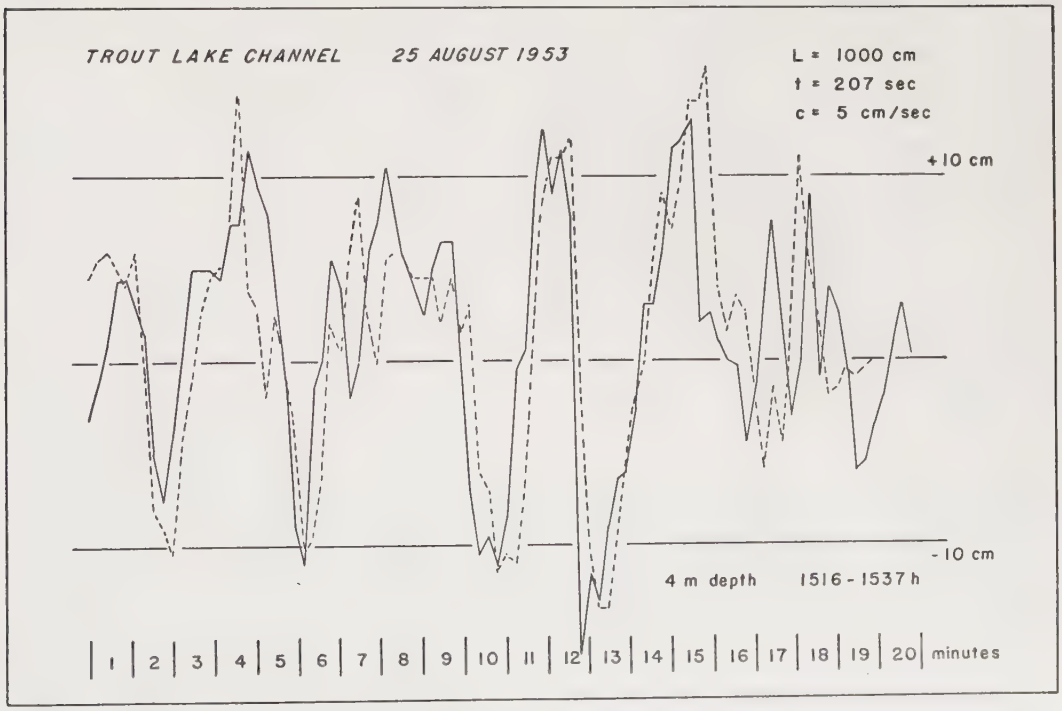

FIG. 6. Variation of temperature at two points separated by a distance of one meter horizontally in Trout Lake Channel at depth of 4 m on 25 August 1953. The solid line denotes the southerly point; the dashed line, the northerly.

TABLE 3. *Internal wave measurements in Trout Lake Channel*

Time of record	Depth (m)	Period, t (sec)	Speed, c (cm/sec)	Wave-length L, (cm)	Direction of Progress of waves (°)	Calculated minimum period by Groen's theory (sec)
1516–1537	4	207	5.00	1035	45	259
1558–1619	4	141	3.85	544	45	185
*1637–1651	4.5	143				172
	5.5					

* Thermopiles oriented vertically to measure "lean" of the waves.

zontally and oriented parallel to the channel was used.

A typical record obtained is shown in Figure 6.

Three separate wave records obtained at this station on August 25, 1953, are summarized in Table 3. There was a 15 mph wind from the south, and the internal waves were travelling downwind.

On the basis of Groen's theory, the minimum wave periods were calculated from temperature soundings taken during each internal wave record. Comparison of the theoretical minimum periods with the observed periods (Table 3) shows that the observed values were consistently lower than the theoretical ones. Since this situation was never observed in Lake Mendota, there is a possibility that the location of the station affected the results. One explanation of this anomaly might be that the internal waves being measured in the Trout Lake Channel originated at some other location where the stability of the water column was greater thereby giving rise to waves of shorter period. The conservative nature of wave period would tend to support this hypothesis.

BREAKING OF INTERNAL WAVES

The thermocline represents the region of maximum stability and therefore the region where mixing through normal turbulence should be suppressed. Wherever density stratification exists, internal waves may occur. Indeed, our measurements have never failed to demonstrate the presence of internal waves when such stratification was present. This suggests that the internal waves may provide the mechanism for the transfer of heat and other properties through the thermocline.

It is generally assumed that one of the symptoms of breaking in waves is increasing asymmetry. While we were not able to observe *increasing* asymmetry, examination of the examples presented in the previous paragraphs shows that truly symmetrical waves are rare. If there is a phase shift in the vertical, there must be either asymmetry of the wave or of the associated stability field. One method for measuring this phase shift uses a bipartite thermopile rig in a vertical position. The phase difference between the two levels then appears directly as a time phase shift in the two records. An example from Lake Mendota is shown in Figure 7. The upper curve led the lower curve by about 12 sec. As the approximate speed for waves of the period observed is 8.5 cm/sec, the waves were leaning forward with a slope of 1:2. Another record from the channel at Trout Lake showed a lean of 1:45. In this case the phase difference of two temperature curves from depths of 3.5 and 4.5 m was approximately 100 sec, and the speed of the waves from measurements at 4 m was about 4.5 cm/sec.

A short term temperature inversion (at temperatures above 4°C) in the presence of internal waves demonstrates the breaking of the wave. Two instances of this phenomenon were observed. The first was observed by a series of bathythermograph casts at one location (Fig. 8). The presence of internal waves is clearly indicated by the periodic fluctuations of the isotherms, especially the 63°F isotherm. One case showed that the 63°F isotherm occurred three times in the same water column with colder water above

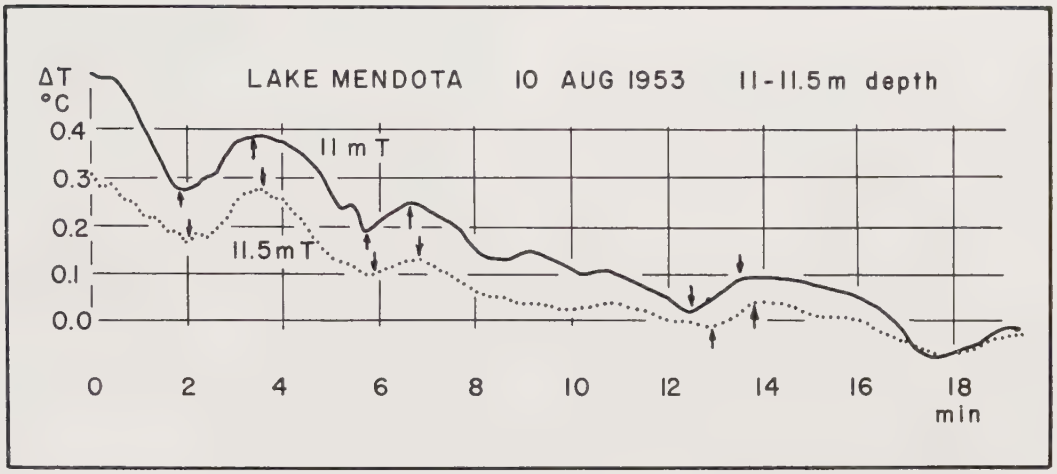

FIG. 7. Variation of temperature at two points separated by a vertical distance of one-half meter in Lake Mendota at 11–11.5-m depth on 10 August 1953. The internal wave crests at 11 m appear to lead those at 11.5 m by about 12 sec.

warmer water. To be sure, the temperature gradient was small where the inversion occurred, but the wave was in the process of breaking and the temperature gradient was gradually being destroyed by mixing.

The second case of a temperature inversion in the presence of internal waves was observed in the course of making direct

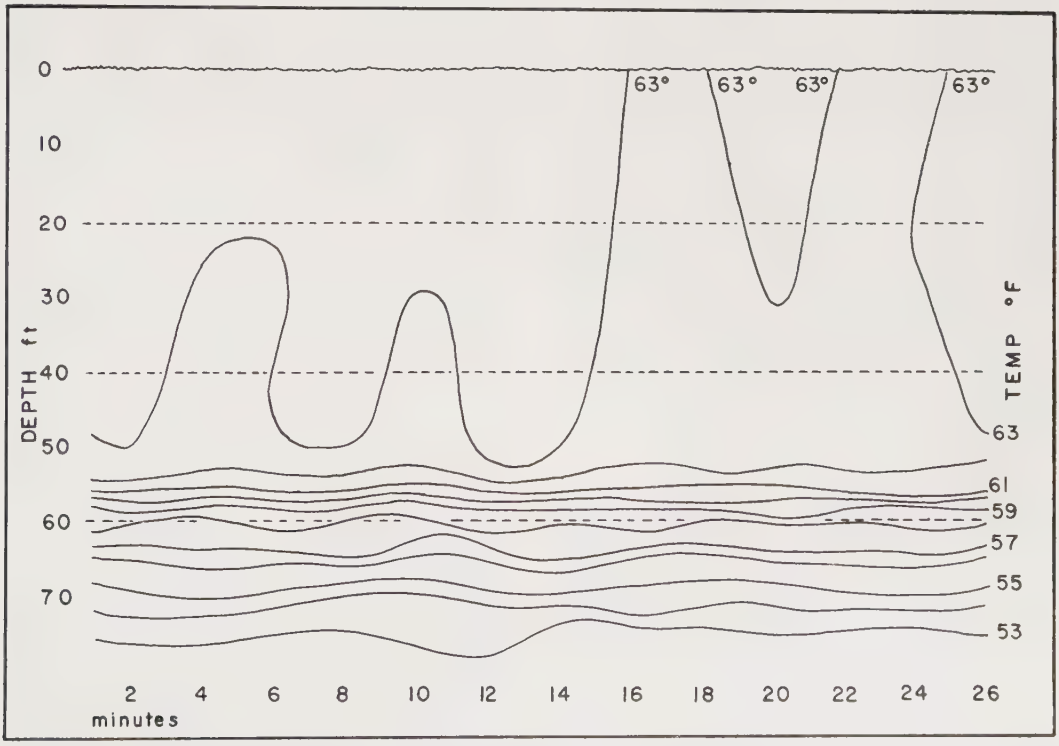

FIG. 8. Variation of temperature with time and depth in Lake Mendota, 7 October 1950. The actual depth of the isotherms was obtained from bathythermograph casts made at 2 min intervals.

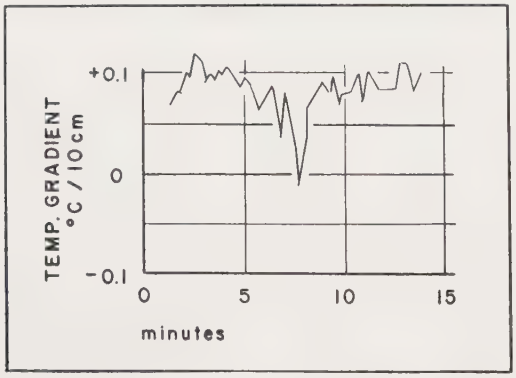

FIG 9. Time variation of the vertical temperature gradient in the Trout Lake Channel at 4-m depth on 25 August 1953.

measurement of vertical temperature gradients. A 5-couple thermopile was connected to measure the temperature gradient through a 10-cm stratum. The voltage developed was recorded by the same system described for the tripartite thermopile rig. A record obtained at the 3.95- to 4.05-m level in the channel at Trout Lake is shown in Figure 9.

Measurements immediately before and after this record clearly demonstrated internal waves at 3.5 to 4.5 m (Trout Lake section). The temperature gradient per 10 cm fluctuated around 0.1°C except for a short period when the gradient dropped to zero and actually reversed for an instant. The record was continuous so that there could be no doubt of the gradient reversal. Less than one hour later the lean of the waves at this level was found to be 1:4.5. It is highly probable that the negative temperature gradient represented a breaking internal wave.

STANDING INTERNAL WAVES

Commonly referred to as the temperature seiche, standing internal waves are frequently present in Lake Mendota. Measurements of these waves were obtained by two methods: 1) repeated temperature soundings at one location over a period of several days, and 2) measurement of the surface reflection of internal oscillations by the water level recorder used to study the surface seiche.

In one attempt to measure the standing internal waves in Lake Mendota temperature soundings were obtained at 90-min intervals for nearly 4 days with the exception of the hours from midnight to 8 a.m. Even with these lapses in the record it was possible to plot the fluctuations of the isotherms and to detect some periodicity in their movements. The thermocline did not rise and fall as a unit; rather, each isotherm behaved slightly differently from all the rest indicating the complexity of the temperature seiche. The most pronounced fluctuations occurred in the isotherms nearest the bottom and the surface. There was an indication of a nodal surface near the middle of the water column. It has been shown by Proudman (1953) that in a stratified medium, which also possesses horizontal velocity shear there should be both a vertical and horizontal wavelength, *i.e.*, nodes such as the one described above.

A standing internal wave will give a reflection on the surface of the lake as a small oscillation of the water level. These surface reflections are discernible on the seiche records obtained with the water level recorder (Bryson and Kuhn 1952). The complexity of the internal seiche is masked by this method since only the net changes in density distribution appear as a surface reflection. From these records can be obtained the period (t) and the amplitude of the surface reflection (η_0). Defant (1940) has shown that the period of a standing internal wave is as follows:

$$t = 2l \sqrt{\left(\frac{\rho}{\rho - \rho'}\right)\left(\frac{h + h'}{ghh'}\right)}$$

Calculation of the period (t) is somewhat unprecise because the values of ρ and ρ' for the layers h and h′ must be approximated from one or two temperature soundings, which are subject to changes due to the standing internal wave. However, typical summer conditions in Lake Mendota give values for t which vary between 10 and 12 hr. The observed values for the period of the standing internal wave are given in Table 4. The amplitude of the surface reflec-

TABLE 4. *Summary of standing internal wave data obtained by the surface reflection records*

Date	Observed t (hr)	Observed η_0 (cm)	Calculated Ξ (cm)
20 July 1951		1.5	1000
20 July 1951	10.8	.15	100
28 June 1953	10.8	.05	45
29 June 1953	10.0	.15	135
30 June 1953	10.5	.10	90
3 July 1953	10.2	.15	83
4 July 1953	10.5	.15	83

tion was shown by Sverdrup (1946) to be as follows:

$$\eta_0 = -\Xi \left(\frac{\rho - \rho'}{\rho}\right)$$

where η_0 is the amplitude of the surface reflection and Ξ is the amplitude of the standing internal wave. Table 4 also shows the observed values of η_0 and the calculated values of Ξ.

Under the influence of a prolonged strong wind the lake surface and the thermocline are forcibly displaced. The first case in Table 4 occurred under these conditions. All the other amplitudes were observed when the lake surface and thermocline were oscillating freely. The usual amplitude appears to be about one meter with occasional very large forced fluctuations.

THE SIGNIFICANCE OF INTERNAL WAVES

Planktonic debris in its slow settling from the upper epilimnion to the bottom of the lake slows up at the thermocline. Oxygen consumption should, therefore, increase at the thermocline, especially in the warmer period. By analogy with the sea there should be an oxygen minimum at that depth. The fact that there is not suggests that the rate of replenishment from above is sufficient to prevent the formation of such a minimum. Is this downward diffusion due to turbudence in the ordinary sense or is it due to breaking internal waves? Our evidence suggests that it may be the latter.

Similarly, as the summer progresses, the portion of the lake occupied by the epilimnion increases at the expense of the hypolimnion. This means a downward flux of heat through or past the thermocline. This could be accomplished either by the breaking of internal waves or by exchange with the portion of the bottom which is alternately in contact with the epilimnion and the hypolimnion as a result of the internal seiche. In a study on heat flux in Lake Mendota, Dutton and Bryson (1959) have found that this latter mechanism can account for a significant portion of the heat transfer through the thermocline.

CONCLUSIONS

1. Both standing and progressive internal waves exist in the lakes which were studied.
2. In general, progressive internal waves in lakes have periods on the order of a few minutes.
3. Both progressive and standing internal waves are generally complex, variable in amplitude, direction, and period, and frequently polynodal both vertically and horizontally.
4. Internal waves probably play a role in the vertical transfer of heat and other variables in a stratified lake.

REFERENCES

BRYSON, R. A. 1948. Disturbances in the Easterlies. Ph.D. Dissertation, University of Chicago.

———,AND P. M. KUHN. 1952. On certain oscillatory motions of lakes. Lakes Invest. Comm. Rep. No. 5, Dept. of Meteorology, Univ. of Wis., 10 pp.

DEFANT, A. 1932. Die Gezeiten und inneren Gezeitenwellen des Atlantischen Ozeans. Deutsch. Atl. Exped. Meteor. 1925–27, Wiss. Ergeb. Bd. **7** Heft. 1.

———. 1949. Uber interne Gezeitenwellen und ihre Stabilitäts bedingungen. Arch. für Met. Geophys. Bioklim., **1**: 39–61.

———. 1940. Die ozeanographischen Verhaltnisse wahrend der Ankerstation des "Altair" am Nordrand des Hauptstromstriches des Golfstroms nordlich der Azoren. Ann. D. Hydrogr. u. Mar. Meteor., November Beihaft, 4. Lief, 35 pp.

DUTTON, J. A., AND R. A. BRYSON. 1959. Heat flux in Lake Mendota. Univ. of Wis., Dept. of Meteorology, Tech. Rep. No. 2 under Contract Nonr 1202(07).

EKMAN, V. W.,AND B. HELLAND-HANSEN. 1931. Measurements of Ocean Currents. Kungl. fysiog. Sallskap, Lund Forhandl. **1**(1).

FJELDSTAD, J. E. 1933. Interne Wellen. Geofysiske Publikasjoner, Oslo, **10**(6), 53 pp.

GROEN, P. 1948. Contribution to the theory of internal waves. Konink. Nederl. Met. Inst. Ser. B., **2**(11), 23 pp.

HELLAND-HANSEN, B., AND F. NANSEN. 1909. The Norwegian Sea. Norweg. Fish. and Marine Invest. Rep., **2**(2) Pt. 1.

———, ———. 1930. Physical Oceanography and Meteorology. *Michael Sars* North Atl. Deep-Sea Exped. 1910, Rep. Sci. Res. **1**.

LATHBURY, A., R. A. BRYSON, AND B. LETTAU. 1959. (Unpublished manuscript.)

LEK, L. 1938. Die Ergebnisse der Strom-Serienmessungen. Snellius Exped. in the eastern part of the Neth. East-Indies 1929–30, **2**, Pt. 3.

NEUMANN, G. 1949. Stabilitätsschwingungen und die innere thermische Unruhe im Meer und in der Atmosphäre. Deutschen Hydrograph Zeitschr., **2**: 52–67.

PROUDMAN, J. 1953. Dynamical Oceanography. New York, Wiley and Sons, 409 pp.

SEIWELL, H. R. 1937. Short period vertical oscillations in the western basin of the North Atlantic. Papers in Phys. Ocean., **5**(2), pp. 44.

STOKES, G. G. 1847. On the theory of oscillatory waves. Trans. Cambridge Phil. Soc., **8**: 441.

SVERDRUP, H. U., M. W. JOHNSON, AND R. H. FLEMING. 1946. The Oceans. New York, Prentice-Hall, 1087 pp.

UFFORD, C .W. 1947a. Internal Waves in the Ocean. Trans. Amer. Geoph. Union, **28**: 70–86.

———. 1947b. Internal Waves Measured at Three Stations. Trans. Amer. Geoph. Union, **28**: 87–95.

———. 1947c. Theory of Internal Waves. Trans. Amer. Geoph. Union, **28**: 96–101.

WEDDERBURN, E. M., AND A. M. WILLIAMS. 1911. The Temperature Seiche. IV. Experimental verification of the temperature seiche theory. Trans. Roy. Soc. Edinburgh **47**: 636–642.

SOME OBSERVATIONS OF CURRENTS IN THE HYPOLIMNION OF LAKE MENDOTA

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ABSTRACT

Observations of hypolimnetic currents in Lake Mendota, integrated over entire seiche periods, indicate that steady motions exist below the thermocline. Seiche currents and turbulence are shown to be insufficient to explain the observed currents.

Hutchinson (1957, p. 287, p. 341) has suggested that deep water currents, at least in medium-sized stratified lakes, are primarily due to internal seiches. He also quotes Mortimer (1953) to the same effect. On the basis of the small amount of published data on the observed currents below the thermocline and the fact that both the usual "surface" seiche and the internal seiche must involve readjustment of mass distribution throughout the entire lake, such a conclusion might be reached on theoretical grounds. It is the purpose of this paper to examine the role of the seiche in producing hypolimnetic currents utilizing statistical data on such currents observed in Lake Mendota.

THE OBSERVED CURRENTS IN LAKE MENDOTA

During the past 10 years, about 2,000 current observations have been made in Lake Mendota. All were made by the "free drag" method, *i.e.*, by observing the displacement of a vane or drag suspended at the desired depth and allowed to drift with the current. This method, which may be used to obtain currents integrated over any time interval desired, is more sensitive to small current velocities than the finest propeller type current meters.

The bulk of the current measurements made in Lake Mendota[1] are summarized in Figure 1. Most of these data were obtained with "drags" half a meter square, and so indicate the mean current in a layer 0.5 m thick centered at the indicated depth from the surface. In the 34 cases indicated for the layer 0–0.25 m, drags both 25 cm and 10 cm high were used.

As might be expected, the mean and modal velocities at the surface of the lake are larger than at a depth of 1 m as indicated when the data from the 0–0.25 m and 0–0.5 m layers are compared to those at 1 m. (Table 1.)

Surprisingly, the currents at 2 m do not fit the general pattern of decreasing mean with increasing depth. Furthermore, the number of currents in the interval 0–0.99 cm/sec might be expected to increase with depth, and generally does except for the striking anomaly of the 2-m depth. These features suggest some cause other than the usual wind drift explanation of the near-surface currents, possibly a larger than expected role of that level in the seiche circulation.

Most of these current measurements were made in midsummer when the thermocline is generally at a depth of 5 to 8 m and generally less than 10 m. Thus, the data from 10 m are largely for currents within the hypolimnion, while for greater depths there

TABLE 1. *Mean and modal velocities for currents in Lake Mendota*

Depth	Mean cm/sec	Mode cm/sec	Number of cases
0–0.25 m	9.36	6–7 (10–11)	34
0–0.5 m	5.93	4–5	107
0.75–1.25 m	4.48	1–2	583
1.75–2.25 m	4.71	3–4 (1–2)	367
4.75–5.25 m	3.23	1–2	255
9.75–10.25 m	2.78	2–3	248
14.75–15.25 m	1.67	0–1	26
19.75–20.25 m	7.7	not defined	10

[1] Data from the entire lake are included, though most are from the southwestern portion.

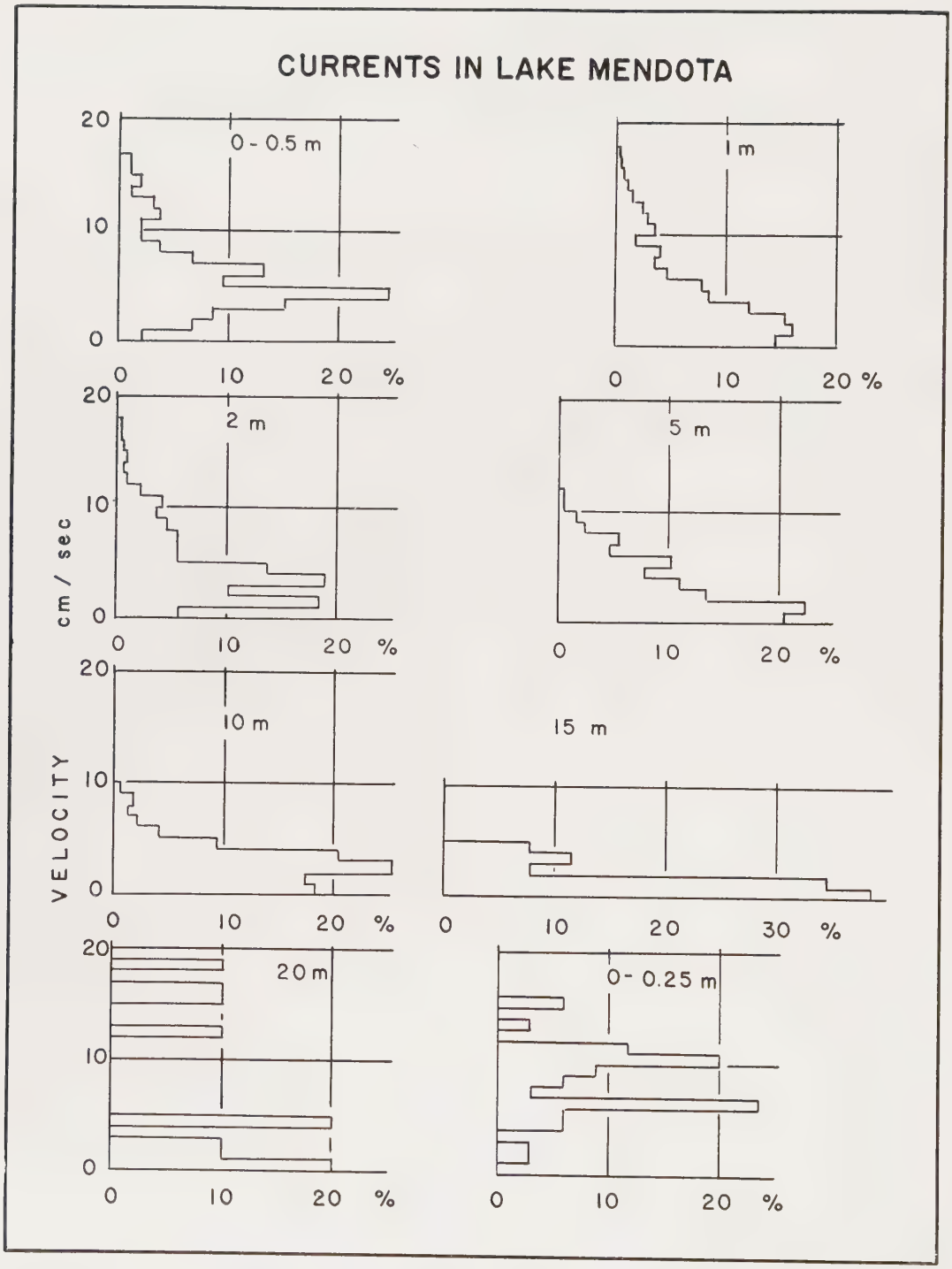

FIG. 1. Frequency distribution of observed current velocities in Lake Mendota. Number of cases for each depth as given in Table 1.

are essentially no cases for which this is not so.

DISCUSSION

While Hutchinson's (1957) discussion of the hypolimnetic currents in lakes suggested that they were largely related to the internal seiche, it is pertinent to inquire whether the usual seiche associated with tilting of the surface of the lake might not also be of significance. After all, if the surface of the lake rises at one end and falls at the other, it is necessary for a certain volume of water to be displaced laterally.

According to Proudman (1953, p. 226), the maximum velocity at the seiche node varies directly with the seiche amplitude and with the square root of the gravity to average depth ratio.

$$V_{max} = A\,(g/d)^{1/2}$$

where V_{max} is the maximum velocity at the node, A the seiche amplitude, g gravity, and d the average depth of the lake. Lake Mendota has an average depth of about 13 m and a typical seiche *range* of 2 to 4 mm. This means a seiche *amplitude* of 1 or 2 mm. The average seiche current will be about half the maximum, or about 0.1 cm/sec at the node, and still less at an arbitrary point in the lake. Seiches up to 10 cm range have been observed in Lake Mendota, but with winds which would preclude current observation. While the methods used to measure currents in Lake Mendota can readily detect currents of this magnitude, Figure 1 shows that the bulk of the currents are an order or two of magnitude larger than might be expected to be associated with the surface seiche.

Still another test might be used to see whether the currents observed within the hypolimnion are seiche currents. If they are, then they should be at least roughly periodic with a period the same as that of the seiche, or 25–26 min in the case of Lake Mendota. Periodic motions may be readily removed by integration over the entire length of the period. Thus, if the water at 15-m depth simply moves to and fro in 25 min, it should return to its original position in that length of time and the net displacement of the measuring drag should be nil. If turbulence

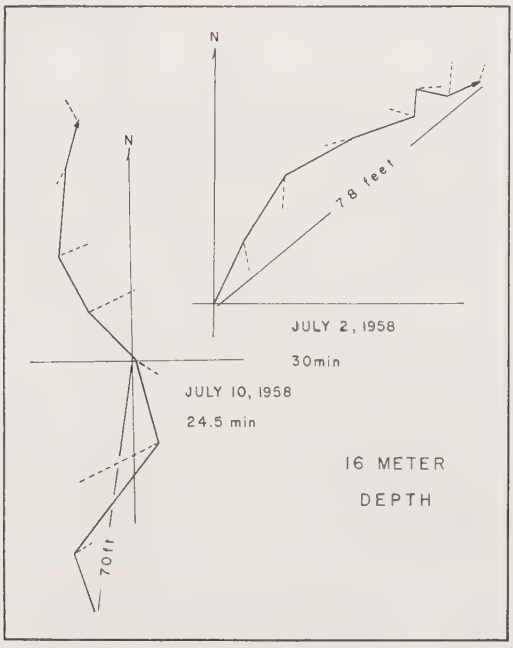

FIG. 2. Typical trajectories of water parcels within the hypolimnion of Lake Mendota during a complete seiche period. The heavy line indicates the path of the parcel and the dashed lines indicate short time departures from the mean current due to the seiche circulation and turbulence. The measurements were made near the node, where maximum seiche currents should have been present.

be added, the net motion may not be nil, but averaged over the entire period should be small compared to the seiche motions. If the net motion is larger in magnitude than the periodic motions, then we must speak of steady motions with superimposed turbulence and seiche currents.

An experiment designed to show what is actually the case was carried out in the summer of 1958. A current drag was suspended within or below the thermocline and its net displacement over an entire seiche period was observed. Figure 2 shows two trajectories of the current drags during a seiche interval. There is indeed evidence of a rather steady change of position from beginning to end of the seiche period, and some sort of periodic or turbulent motion superimposed upon this. However, the latter is small and causes little variation in the trajectory of the drag. In 10 repetitions of the experiment at depths of 13 to 16 m, the drag

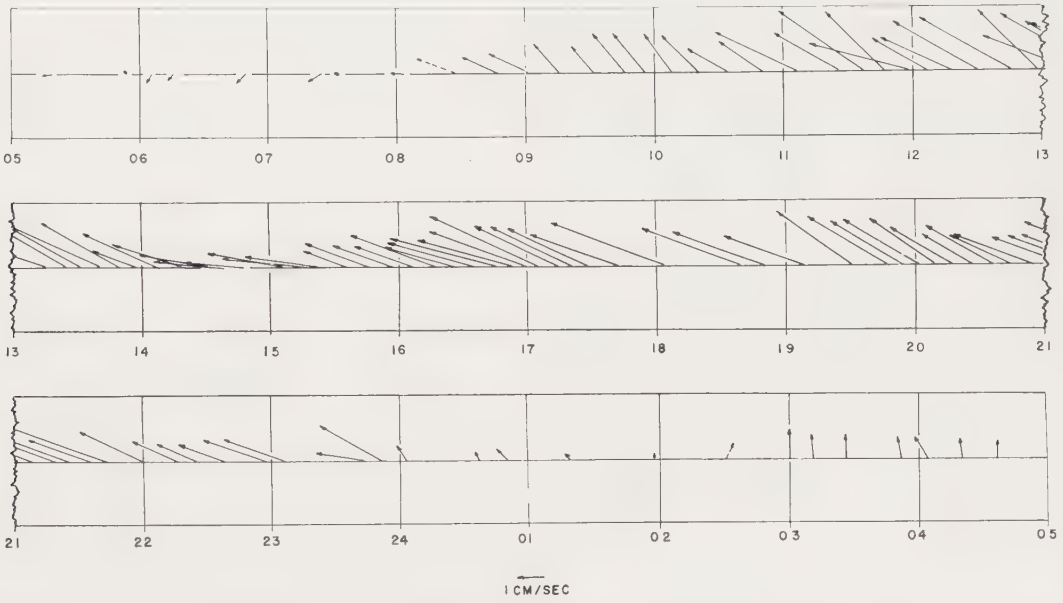

FIG. 3. Currents observed at a depth of 10 m northeast of Second Point, Lake Mendota, between 0500 on 20 Aug., 1954, and 0500 on 21 Aug., 1954. The direction of the arrow indicates the set of the current, north being at the top of the page. Dashed arrows were uncertain as to speed. A diurnal variation is apparent, but there is clearly a mean flow larger than any superimposed oscillations due to the internal seiche. This location was very near the known seiche node, and several meters below the thermocline.

never returned to its original position as it must if the currents are indeed seiche currents, nor for that matter did it return to near its original position. In fact, in no case did the current show a reversal of direction. From this we must conclude that the currents within the hypolimnion are not primarily associated with the surface seiche.

Although the velocities for intervals of 3 or 4 min computed from the trajectories shown in Figure 2 show a moderately large variability, most of the currents tabulated in Figure 1 are means for periods of 5 to 10 min, and thus may be regarded as more stable and representative than those computed in connection with this experiment. The velocities given in Figure 1 cannot then be regarded as samplings of an essentially turbulent flow or a random selection of seiche currents unless those currents be of longer duration than those associated with the surface seiche.

The area of Lake Mendota at the summer thermocline depth on one side of the nodal line is about 14×10^6 m^2. For an internal seiche range of 5 m, the thermocline under this half of the lake must move vertically an average distance of about 2.5 m in about 5 hr, which is about half a seiche period. Since the displaced hypolimnion must move through about 6×3000 m^2 of nodal plane, the average current through the nodal plane is on the order of 8–12 cm/sec, and the mean internal seiche induced current might be 4–6 cm/sec. This is probably a high figure, for the average internal seiche range is probably less than 5 m. Most of the currents observed within the hypolimnion are indeed of the right order of magnitude to be due to internal seiches. However, during the summer of 1954 currents were measured every few minutes for a period of nearly 24 hr, *i.e.*, for about two internal seiche periods (Fig. 3). The results of this experiment were the same as that described above, for while the individual current vectors varied from time to time, they were predominantly from one direction. If the currents within the hypolimnion are due to the internal seiche motions, then the resultant current

vector over an entire seiche period should be nil, or nearly so. Thus, it can be concluded that there are quasi-steady currents within the hypolimnion which are not due to internal seiches, and which are not negligible in magnitude.

SUMMARY

The data and the experiments described in the preceding paragraphs do not rule out the existence of seiche currents or turbulent motion within the hypolimnion. However, they do strongly indicate that there are other causes which are more important in the production of hypolimnetic currents than seiches. The variation of the current vectors during the course of the seiche interval, while not found to be strictly periodic, may very well be due to the seiche, both uninodal and complex. It may seem, however, that it is best to regard the deep currents as quasi-steady, with superimposed variations due to seiches. There remains the problem of elucidating the relative magnitudes of those currents due to the thermally induced pressure gradients and those due directly to the wind.

REFERENCES

HUTCHINSON, G. E. 1957. A treatise on Limnology, Vol. I—Geography, Physics, and Chemistry. John Wiley and Sons, Inc., New York. 1015 pp.

MORTIMER, C. H. 1953. The resonant response of stratified lakes to wind. Schweiz. Z. Hydrol., **15**: 94–151.

PROUDMAN, J. 1953. Dynamic Oceanography. Methuen and Co. Ltd., London. 409 pp.

TEMPERATURE VARIATIONS IN ESQUIMALT LAGOON—A SMALL LANDLOCKED BODY OF WATER SUBJECT TO TIDAL FLUSHING[1]

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ABSTRACT

Time series observations, extending over two tidal cycles, of temperatures and micro-temperature fluctuations at different positions and surface wave amplitudes at two fixed locations have been obtained in Esquimalt Lagoon (Esquimalt, B.C., Canada). The Lagoon is coupled to the sea by a narrow channel which permits about one-third of the low tide water to be exchanged during a tidal cycle. A technique using drags and aerial photographs has enabled the water circulation to be measured. Temperatures measured two inches from the bottom exhibit remarkable stability ($<0.1°C$ fluctuation). No correlation between vertical temperature structure and surface waves or between micro-temperature fluctuations and surface waves is observed.

INTRODUCTION

Temperature and surface wave amplitude measuring equipment was installed in the Esquimalt Lagoon near Victoria, B.C. in connection with a sound propagation study (Scrimger 1959). The facilities permit rapid measurement of water temperature at a location near the center of the water area, water temperatures in the inlet channel to the lagoon, and surface wave amplitude. The surface wave recorders also serve as accurate ($\pm$ ¼ inch in 10 ft) depth gauges. All measurements are recorded remotely at the base laboratory situated at the north end of the lagoon. A complete description of the installation has been given by Scrimger *et al.* (1959). The observations give a sufficiently interesting picture of the temperature structure of the lagoon, and the factors which influence it, to merit reporting separately from the sound propagation work.

The lagoon is connected to the sea by a narrow, shallow channel about 600 ft in length, through which the inward flow is essentially turbulent and the outward flow laminar. This coupling to the sea permits an exchange of water, in the course of a tidal cycle, of the order of 35% of the enclosed volume at low tide. Temperatures in the lagoon water are thus influenced by an inrush of well-mixed isothermal and isosaline water. The tidal mixing in the lagoon thus differs from some other landlocked water bodies, for example Dabob Bay, (Garrison and Shaw 1956), where the mixing processes are governed largely by water density.

Three time series measurements extending over approximately two tidal cycles (24 hr) have been obtained, two in the summer months of 1958 and one in the winter in January, 1959. These permit a comparison on a time scale to be made between a) vertical temperature structure and microtemperature fluctuations near the middle of the water area, b) temperature and flow direction of water in the channel connecting the lagoon and the sea, c) surface wave amplitude, and d) water depth at a fixed position in the lagoon.

Examination of the results permits some definite conclusions to be drawn concerning the mixing processes effective in the lagoon as tidal flushing occurs. In particular, attention has been given to the effect of surface waves on vertical temperature structure and microtemperature fluctuation, and to the amplitude of small temperature fluctuations at different depths. Water circulation in the area has been measured. A technique using drags at different depths,

[1] Contribution from the Pacific Naval Laboratory, Esquimalt, of the Defense Research Board of Canada. The assistance of R. Chappell and D. Street in making the measurements and analysing the data, and the many helpful suggestions of Drs. W. N. English and R. W. Stewart are gratefully acknowledged.

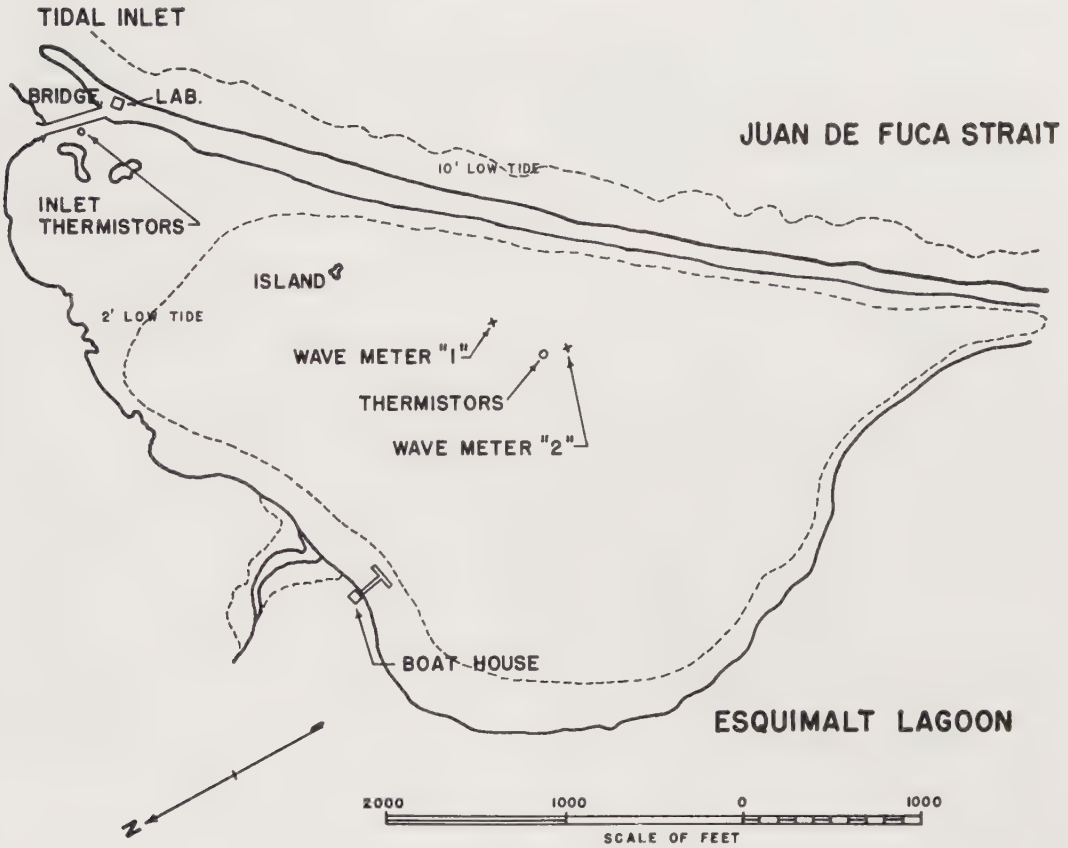

FIG. 1. Diagram of the Lagoon showing the position of the monitoring equipment.

the surface markers for which are photographed from the air, has been applied successfully.[2]

METHODS

Measuring Equipment

Figure 1 shows the location of monitoring devices. Five thermistors, mounted at about 2-ft intervals on a vertical support (the lowest thermistor being 2 in. from the bottom) serve as temperature sensing elements for the recorder housed in the base laboratory. Any one of the 5 thermistors can be selected to display temperature linearly on chart paper in the range of 2–22°C with an accuracy of ± 0.2°C. Amplification of the thermistor bridge output voltage enables microfluctuations as small as 0.005°C to be observed with a response time of 1 sec, in which case the absolute value of temperature is not known.

Two thermistors have recently been installed at 6 and 42 in. from the bottom in the inlet channel. An electronic switch is used to switch periodically between bridge outputs enabling temperatures from both thermometers to be displayed on a Varian chart recorder. The output associated with either thermometer is identified by the switching period.

Each wave amplitude recorder consists simply of a light float coupled mechanically to a potentiometer, the output voltage of which is dependent on the relative height of

[2] This method is suitable for measuring water circulation on a larger scale in cases where conventional current meters cannot provide the required horizontal coverage or direction of low velocity flow. Alternatively, reflectors or coded transponders used in conjunction with a fixed location radar transmitter could be used instead of the aerial photographic technique.

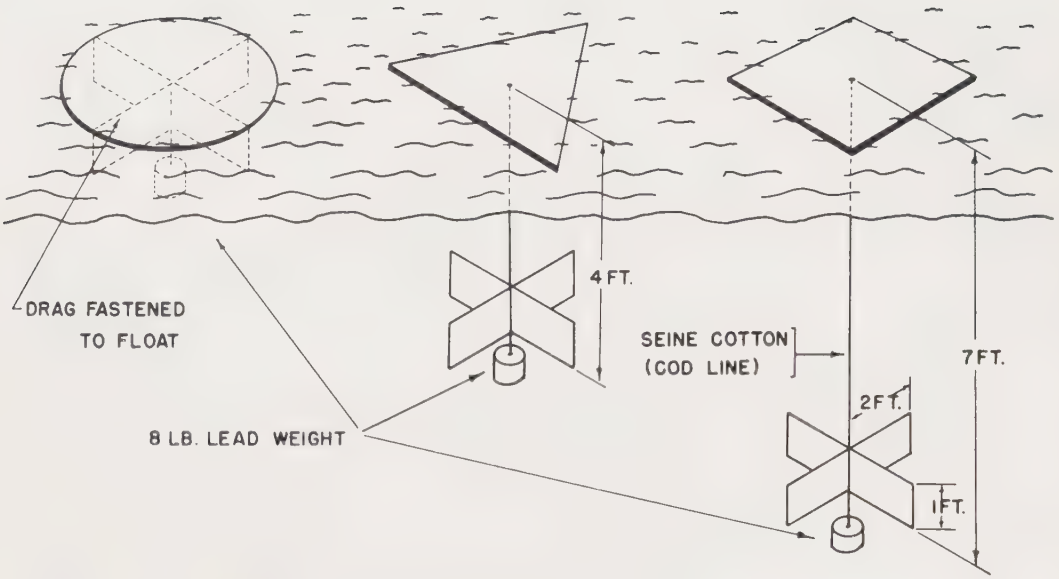

FIG. 2. Diagram of the drags and surface markers.

the float. Minimizing the inertia of the moving parts enables wave amplitudes from ¼ to 8 in. crest to trough to be recorded continuously and linearly to within 2% of full scale.

The drags used for the current measurements were made from two pieces of ⅜-in. plywood, joined such that four mutually perpendicular, rectangular vanes measuring 1 ft × 2 ft were suspended in the water with their planes vertical as shown in Figure 2. The overall horizontal dimension of the drag was thus 4 ft with a vertical dimension of 1 ft. The drags were suspended from surface markers, made from ⅜-in. plywood painted with "Dayglo Arc-Yellow" fluorescent paint. As the surface markers were awash, influence of wind was negligible.

Temperature Observations

Temperature microfluctuations were observed consecutively for a 5-min period at each of the 5 thermistor positions at the central location. A set of observations of temperature fluctuations at all 5 depths as shown in Figure 3 thus took about 25 min. A temperture profile was then obtained by recording the temperature at each of the 5 thermistor levels for a few seconds. This sequence of observations was repeated throughout the 24-hr observation period so that temperature profiles were obtained every ½ hr and microfluctuations recorded consecutively at the 5 depths during the intervening time. Wave amplitudes and water depth at the wave monitor positions were recorded continuously. Water temperatures in the inlet channel were taken every ½ hr using a mercury-in-glass thermometer. (Continuous recording using thermistors—one near the bottom and one at 3 ft from the bottom—was used for the January observations.) Air temperature and visibility were noted at approximately ½-hr intervals, as was also the flow direction of the water in the inlet channel. The relative level of sea surface *outside* the lagoon was taken from the tide tables.

Current Observations

The low current velocities associated with the water circulation in the lagoon precluded the use of conventional current measuring devices. Drags, supported at different depths by plywood sheets shaped to differentiate drag depth, as shown in Figure 2, were therefore used. A time sequence of photographs taken from a helicopter of the surface markers showed the water circulation. Three drags at the surface, three at 4 ft

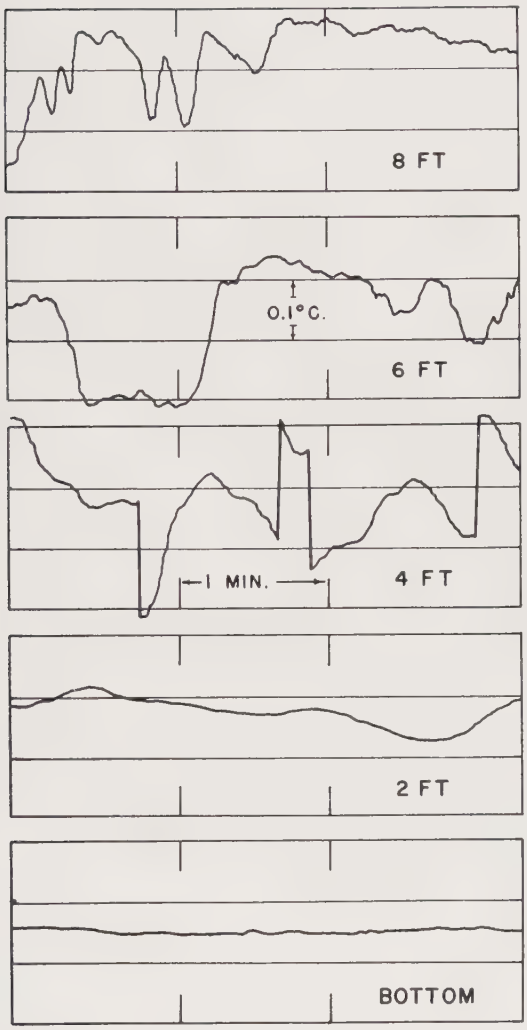

FIG. 3. Tracings of micro-temperature fluctuations observed at the 5 depths—measured relative to the bottom. The lowest, or bottom, thermistor was actually 2 in. from the bottom. Discontinuities in the 4-ft record are due to calibration.

and three at 7 ft, were launched in a group close to the sound transmission range. Using a fluorescent paint and surface markers about 4 ft in width, satisfactory resolution of shape was obtained up to an altitude of 3,000 ft.

RESULTS

Temperature Observations

Observations made in July and August of 1958 and January, 1959, are shown in Figures 4, 5 and 6 respectively. T_1 refers to temperature measured at the upper level, *i.e.* 8 ft from the bottom; T_2 to temperature measured at 6 ft from the bottom, etc. Surface wave amplitude is shown symbolically superimposed on the lagoon water depth plot. Amplitude of temperature fluctuations observed at the 5 chosen levels is shown in Figure 7.

Current Observations

The currents associated with the tidal flushing, as indicated by the drag positions at different times, are shown in Figures 8, 9 and 10. On the outgoing tide (Figures 8 and 9) the exchange is made primarily by the surface and deep water. The flow velocity at the surface is of the order of 0.1 knots and near the bottom of the order of 0.04 knots with the flow direction in both cases being well defined and towards the inlet. The water flow at mid-depth shows a very low velocity and an indefinite direction. The plot of the individual mid-depth drag positions in Figure 9 indicates a stagnation point near the island and flow velocity between 0.01 and 0.02 knots. The currents produced by the flushing on the incoming tide are shown in Figure 10. As for the outgoing tide, most of the water exchange in this case is effected at the surface where the current velocity is about 0.4 knots near the center of the water area, decreasing to 0.1 knot near the southern end. A reverse flow occurs at the bottom and mid-depths. The bottom flow velocity increases from 0.06 knots at the center of the water area to 0.15 knots near the island. The mid-depth velocity averages 0.1 knots.

DISCUSSION

General Characteristics

The effect of tidal flushing on vertical temperature distribution shows up very markedly in the July observations (Figure 5). Three general features are noteworthy in this time series: a) the 1½-hr time delay between the establishment of the inflow of cold (13°C) sea water and the sudden variation in temperatures observed near the central region; b) the sudden rise of tempera-

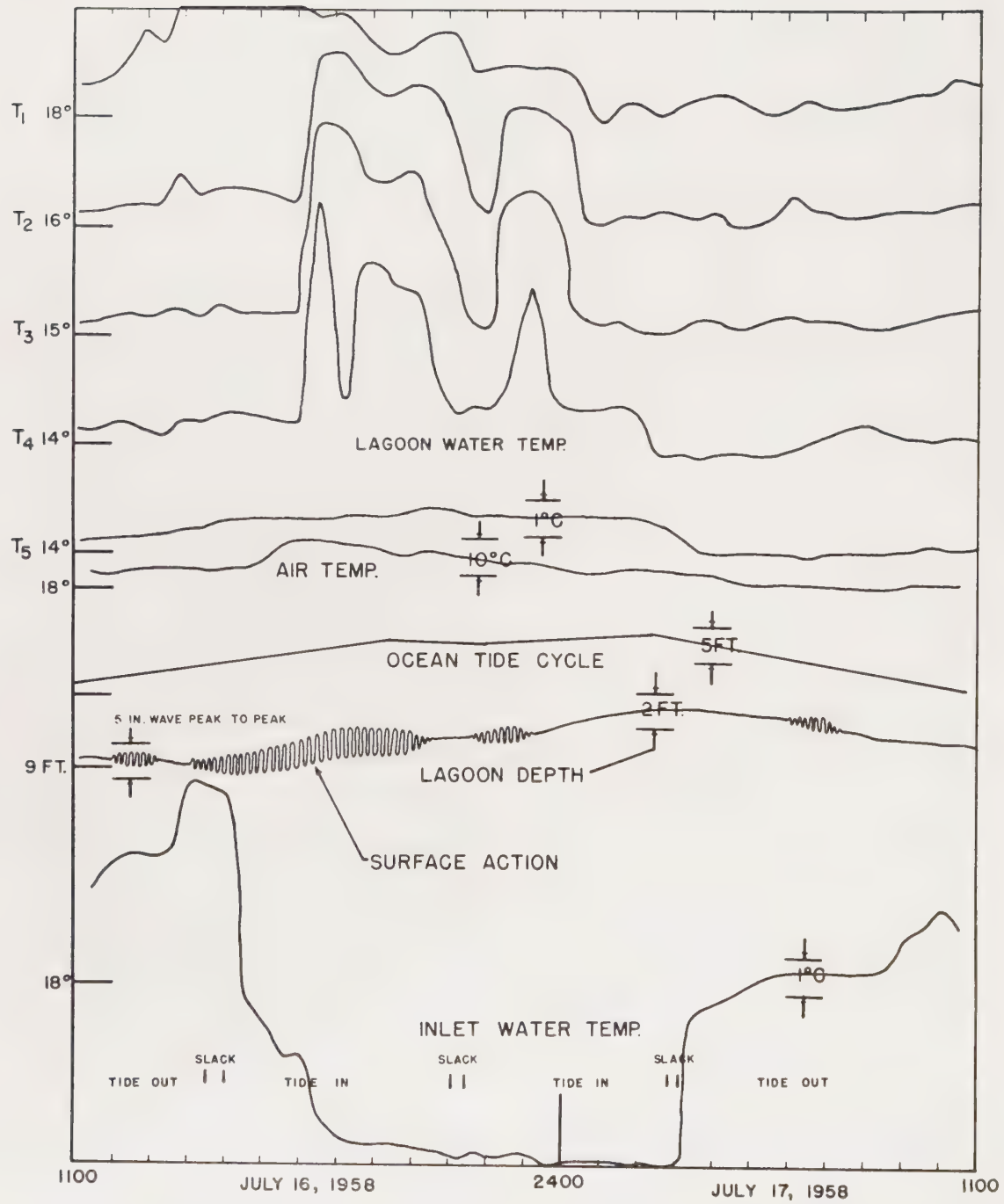

FIG. 4. Temperature and surface wave amplitude measurements made in July, 1958. T_1 refers to the upper (8-ft) water level; T_2 to the 6-ft water level, etc., and T_5 to the bottom water level.

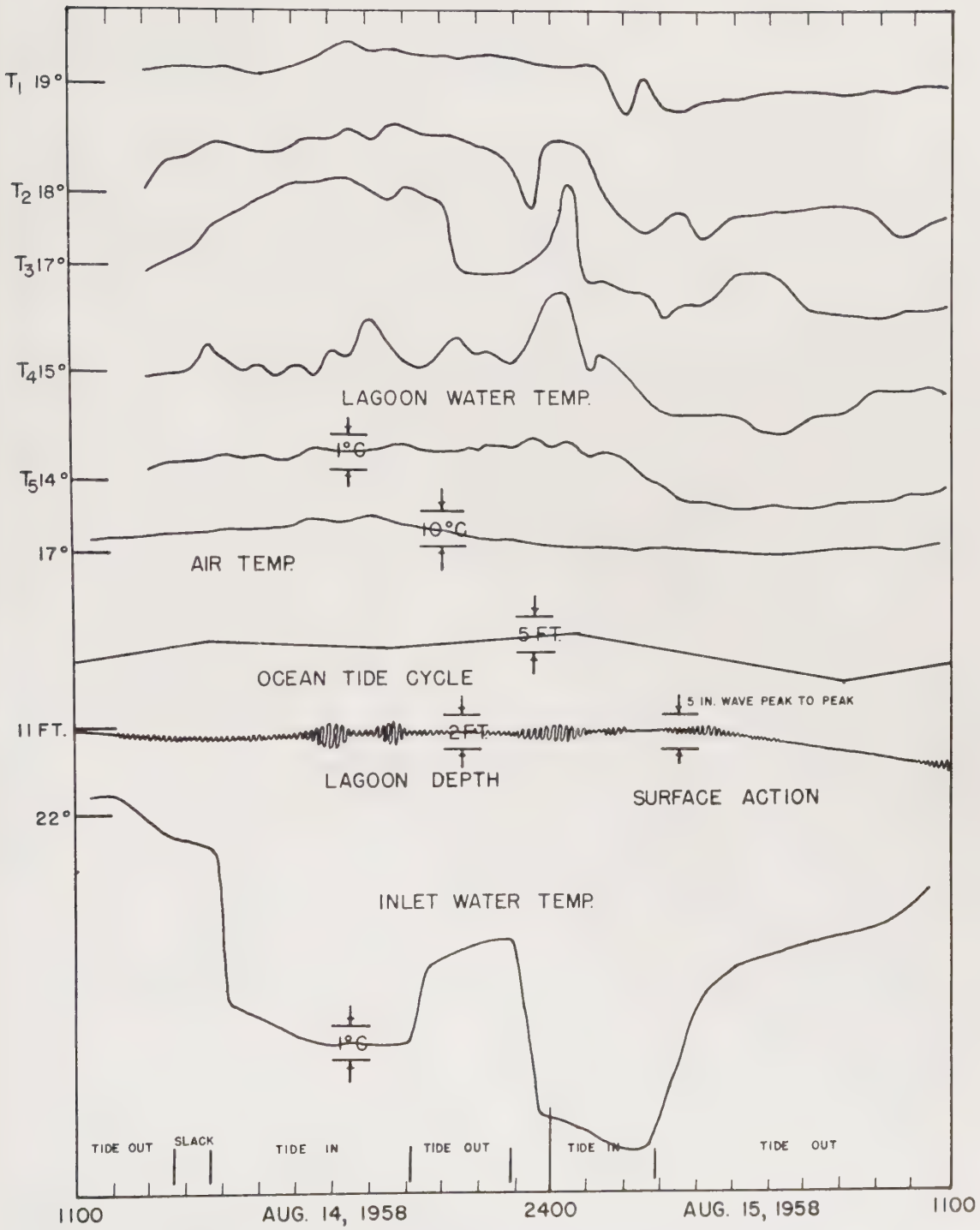

FIG. 5. Temperature and surface wave amplitude measurements made in August, 1958. T_1 refers to the upper (8-ft) water level; T_2 to the 6-ft water level, etc., and T_5 to the bottom water level.

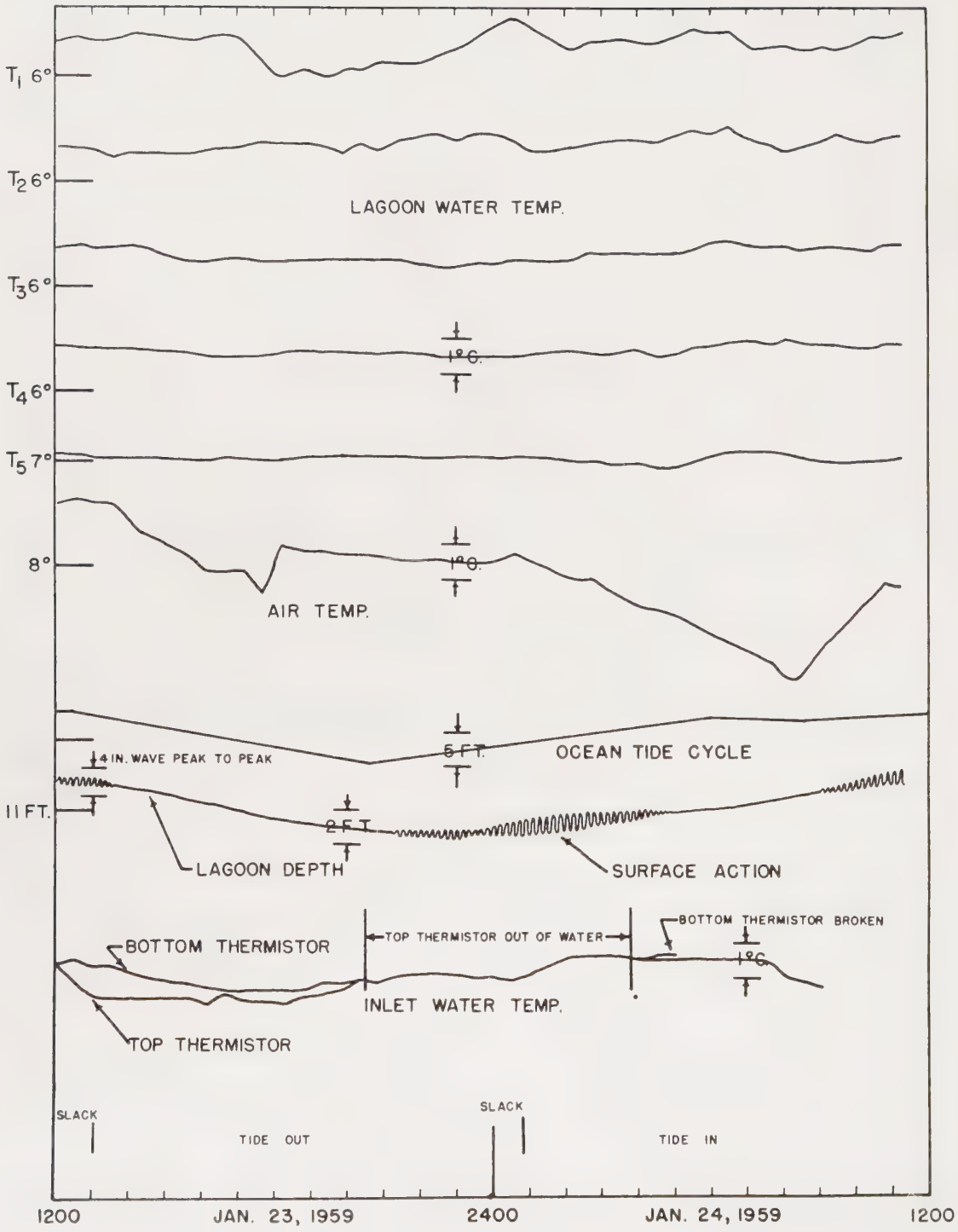

FIG. 6. Temperature and surface wave amplitude measurements made in January, 1959. T_1 refers to the upper (8-ft) water level, T_2 to the 6-ft water level, etc., and T_5 to the bottom water level.

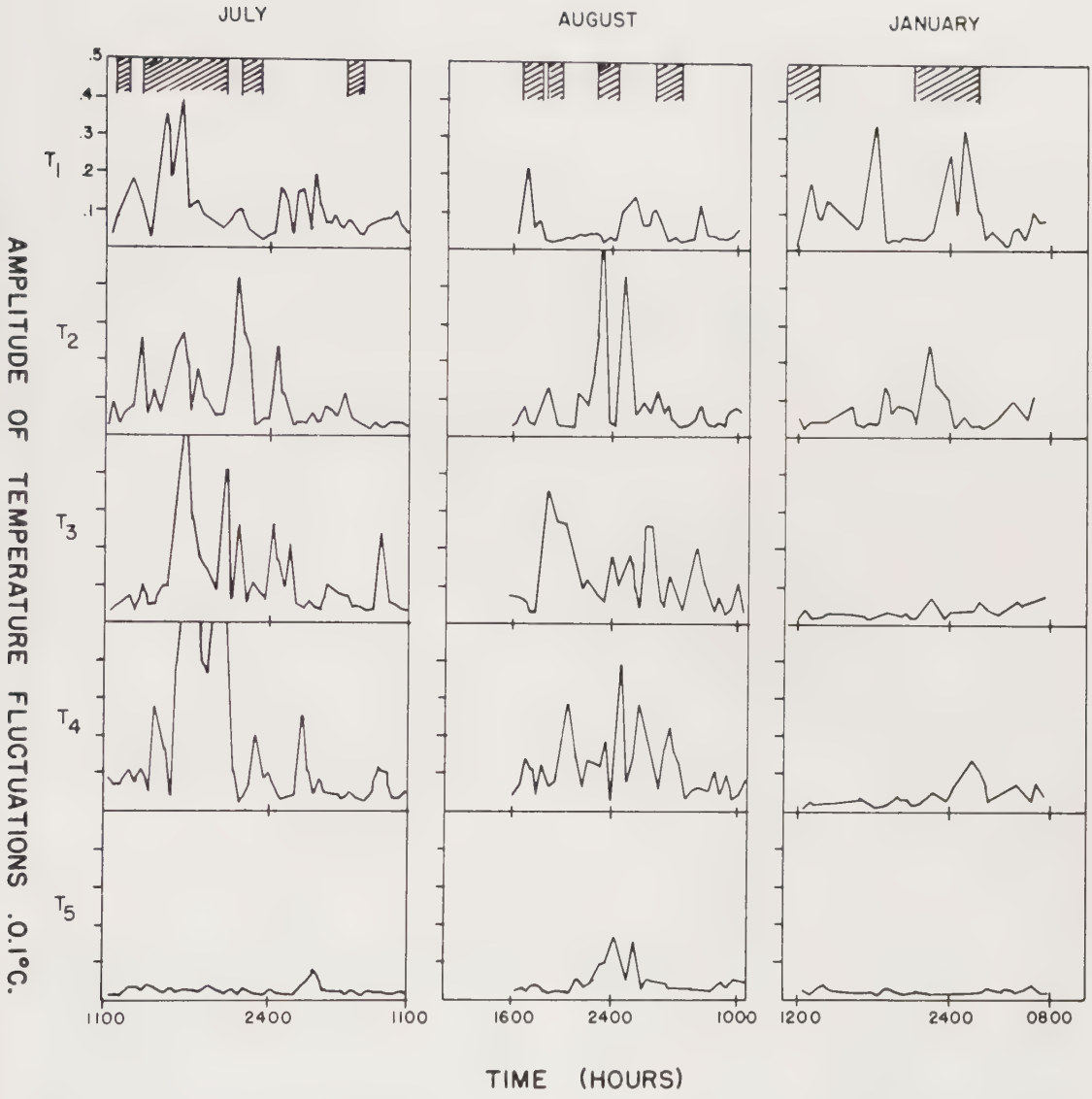

FIG. 7. Amplitude of temperature fluctuation observed in 5-min periods. Shaded sections indicate presence of surface waves.

tures associated with the cold inflowing sea water; c) the stability of the temperature of the bottom few inches of water as indicated by the lowest thermistor. The sudden rise of temperature observed by the three centrally located thermistors at 1700 hr is evidently associated with the warm shallow water at the north end of the lagoon. A warm front appears to have moved towards the thermistor array at between 0.2 and 0.3 knots. This is in fair agreement with the surface current velocity observed for the incoming tide but suggests that on this occasion no reverse current was present at mid-depth and that the inflowing water was well mixed to a depth 2 ft from the bottom. The stable temperature observed in the bottom water layers may be due to a highly persistent, low-velocity outflowing current.

Compared with the July results, the *outside* sea water temperature was essentially unchanged in the August observations (Fig-

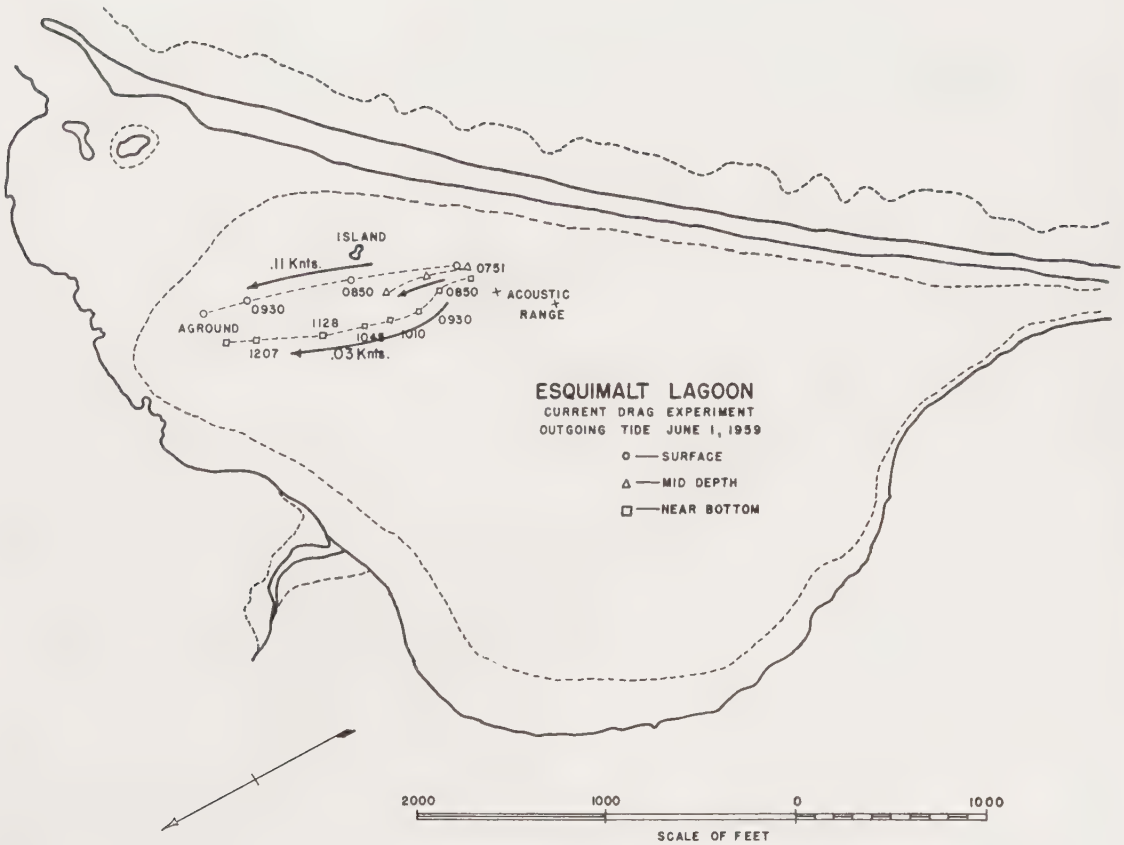

FIG. 8. Drag movement during the outgoing tide. Symbols represent center position of the group of 3 drags at the time shown.

ure 5) but the effects of the tidal flushing are noticeably less pronounced. The vertical temperature distribution shows considerable variation but little correlation with the tidal cycle. In January (Figure 6) the water in the lagoon is nearly isothermal and at the same temperature as the sea water. Tidal flushing has, therefore, negligible effect on temperature distribution. The vertical temperature distribution obtained from the vertically spaced thermistor pair mounted in the inlet channel is interesting. The flow in the channel is laminar during the ebb tide and as can be seen from Figure 6, the surface temperature is, under these conditions, between 1 and ½°C *lower* than that measured at the bottom.

Temperature Microfluctuations

The amplitudes of temperature fluctuations observed during the 5-min periods are shown in Figure 7. The most outstanding feature of these observations is the low level of fluctuation present in the bottom few inches of water although large fluctuations are observed at the level 2 ft from the bottom. In July and August fluctuation amplitudes are large at the 2-, 4- and 6-ft levels and somewhat less near the surface, *i.e.* at the 8-ft level.

Influence of Surface Waves

It is worth-while to consider the extent to which surface waves influence temperature structure. There is a generally accepted belief that surface waves at sea, particularly those associated with storms, are responsible for the destruction of temperature gradients in the top 20 to 100 ft of water. Two possible mechanisms are present: the particle

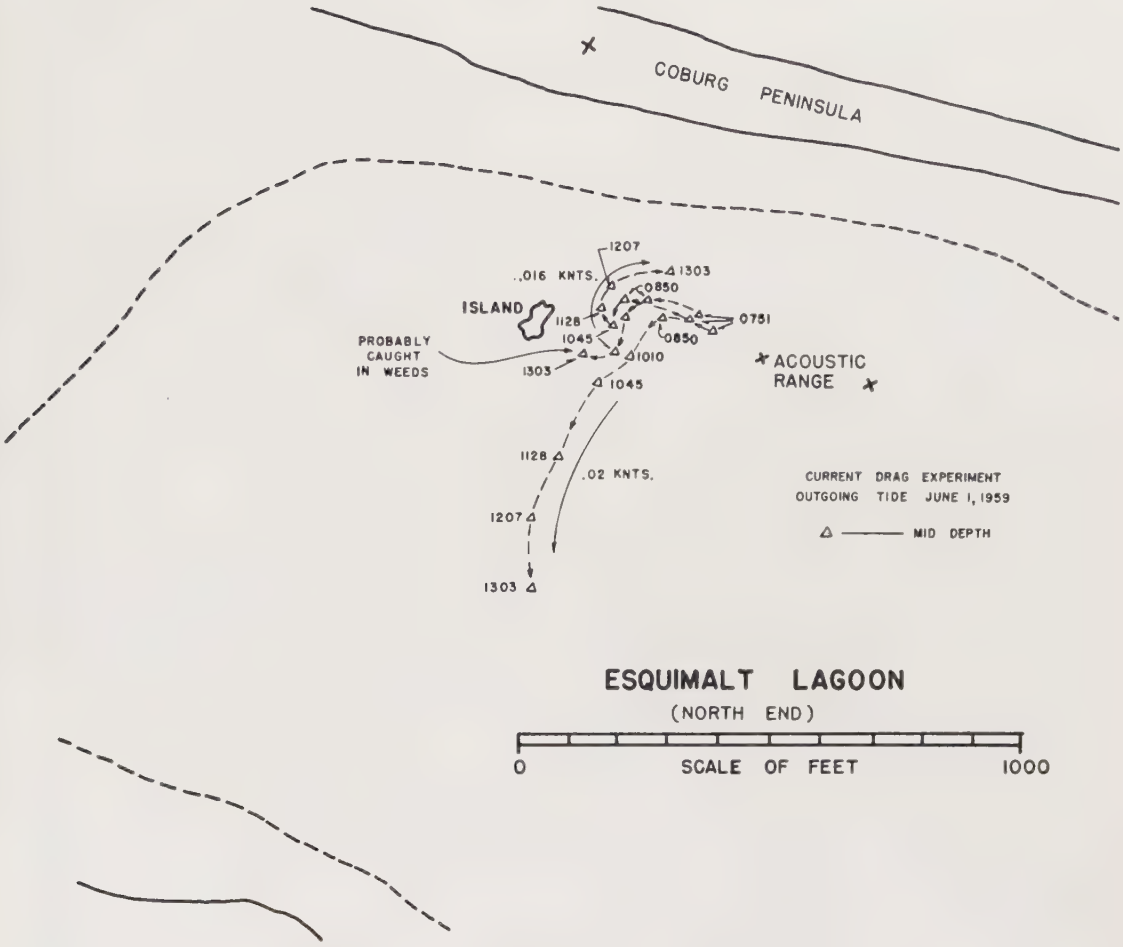

FIG. 9. Movement of mid-depth (*i.e.* 4 ft) drags during the outgoing tide. Due to the lack of a well-defined current these drags gradually dispersed. The track of each drag is therefore shown.

movement associated with the waves, which is a constrained movement, and velocity shear associated with the translation of surface water under the influence of the waves and the generating wind.

Spectral analysis of the wavemeter signals shows the predominant periods of the surface waves to lie between 1.2 and 0.45 sec depending upon amplitude. Neglecting the effects of the generating wind and the bottom, the corresponding wavelength-range for free gravity waves is 1 to 7 ft. Our temperature measurements are to some extent restricted in that temperatures are observed at fixed distances from the bottom and therefore at variable distance from the surface in the course of a tidal cycle. However, considering the wavelength-range of the surface waves and the proximity of the upper thermistors of the array to the surface for a large part of the time, any influence exerted by the surface wave on temperature structure should be apparent.

The periods during which surface waves were observed are shown on the time series and fluctuation amplitude plots. Within the limitations mentioned above no correlation is apparent between surface waves and temperature structure or temperature fluctuation, hence mixing by wave action alone does not seem to occur.

CONCLUSIONS

The vertical temperature structure in a

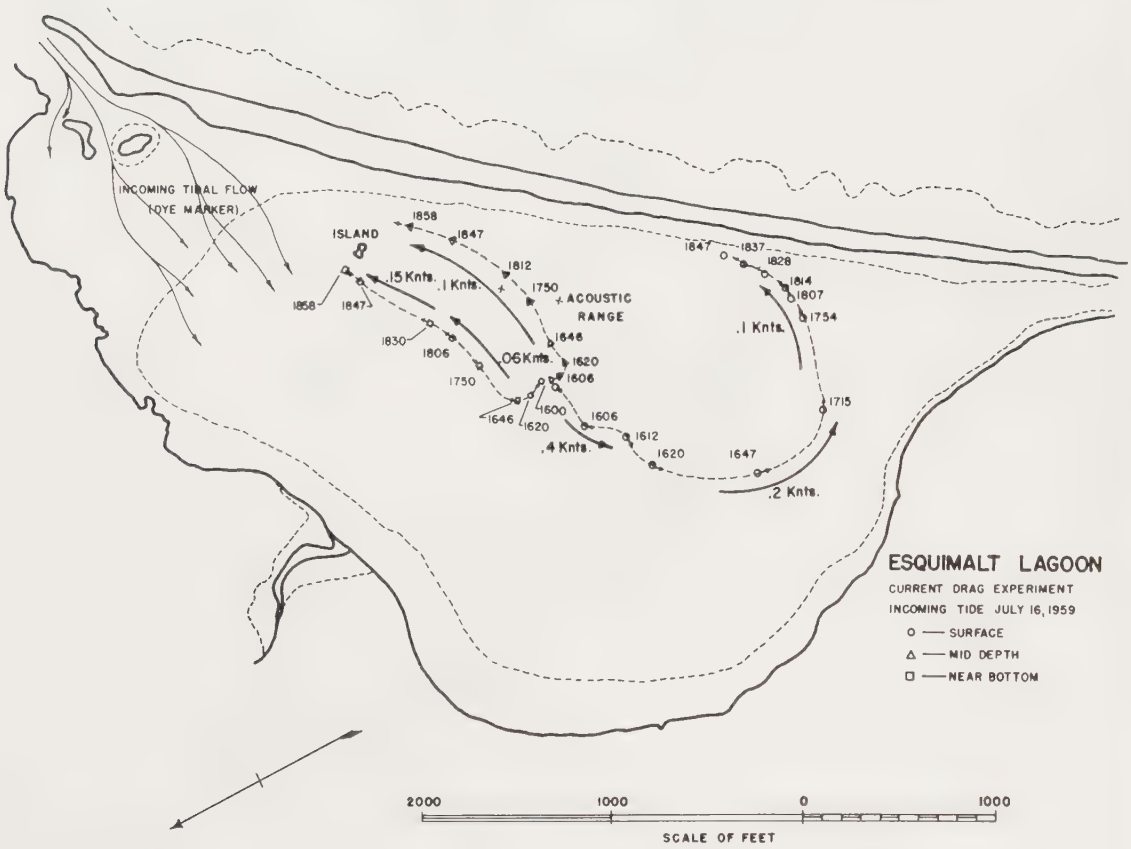

FIG. 10. Drag movement during the incoming tide. Symbols represent center position of the group of 3 drags at the time shown.

shallow enclosed body of water such as Esquimalt Lagoon undergoes large variations which are produced by the influx of cold sea water during the incoming tide. However, there exists a bottom layer of water of a few inches in thickness which exhibits a noteworthy temperature stability. In this layer microtemperature fluctuations of periods up to one minute rarely exceed 0.1°C. The effects of surface waves on vertical temperature structure and microtemperature fluctuations have been examined and there appears to be no correlation between changes in vertical temperature structure or amplitude of microtemperature fluctuations and surface waves. The technique of using drags at various depths, the surface markers for which are photographed periodically from the air, has proved valuable for measuring current distribution and is suitable for use on a larger scale, as for example, in estuaries.

REFERENCES

GARRISON, E. R., AND J. T. SHAW. 1956. Oceanographic measurements in Dabob Bay. Applied Physics Lab., University of Washington, T.E., 56–37, 21 pp.

SCRIMGER, J. A. 1959. Shallow water sound propagation studies in an intermediate scale model. U.S.N. Jour. Underwater Ac., **9**(3): 393–402.

———, D. S. STREET, AND R. CHAPPELL. 1959. An installation for studying sound propagation in shallow water on an intermediate scale. Pacific Naval Laboratory, Tech. Memo. 59–11, 1–8.

CARBAMINO CARBOXYLIC ACIDS IN PHOTOSYNTHESIS[1]

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ABSTRACT

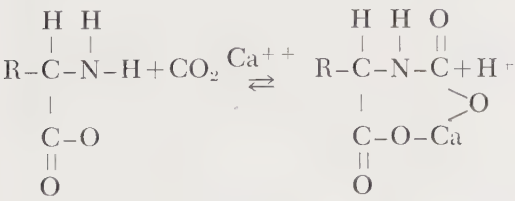
Cultures of *Platymonas* sp., *Nitzschia closterium*, and *Porphyridium cruentum* were found to utilize the carbamino complex of alanine or sea water concentrates in preference to inorganic forms of CO_2 for photosynthesis. Evidence for this was obtained by inhibition of $C^{14}O_2$ uptake of photosynthesizing cells upon addition of the complex. Cell growth and oxygen evolution, however, continued to increase in a normal fashion. By labeling the complex in different portions of the molecule, it was shown that the plants were utilizing the N–carboxylate group directly as a source of CO_2 while utilization of other portions was not evidenced.

Several other organic compounds were tested for response in the algae but only ammonium carbonate, known to contain small portions of ammonium carbamate, acted similarly.

INTRODUCTION

The carbamino derivatives of amino acids have been studied rather extensively by Faurholt (1924), Ferguson and Roughton (1935), Stadie and O'Brien (1936), and Neuberg, *et al.* (1955) as a mechanism for carbon dioxide transport by mammalian blood. Recently Neuberg, *et al.* (1957) gave experimental evidence of the solubilization effects of amino acids and carbon dioxide on calcium carbonate and calcium phosphate in sea water. The primary function of the carbamate is that of complexing calcium and forming the soluble calcium salts of carbamino carboxylic acids.

The carbamino carboxylates form according to the following general equation:



in which R represents the remaining portion of the structure of an amino acid, peptide or protein.

The velocity constant for α and β alanine carbamates has been evaluated by Jensen and Faurholt (1952) to be $10^{4.32}$ and $10^{5.04}$ respectively at 18°C in distilled water. The reaction occurs only when the compounds are in the anion form. Quantitatively higher concentrations are formed at pH values above the isoelectric point of these amphoteric compounds.

Acidic solutions of the carbamates decompose almost instantaneously as does ammonium carbamate. In strongly alkaline solutions (pH 13.0) decomposition occurs giving CO_2 and carbonate. However, in weakly basic solutions there is an equilibrium established between carbonate components and amine-containing groups so that in any such system

$$C_{AMH} + C_{AM} + \Sigma C_{CO_2 \text{ containing components}} \rightleftarrows C_{\text{carbamate}} + C_{AMH} + C_{AM} + \Sigma C_{CO_2 \text{ containing components}}.$$

The velocity constants for the interchange are dependent on the nature of the amine compounds but are in general relatively fast.

Proteins and peptides have a wide range of isoelectric points. In proteins proton exchange is concentrated at the three pH zones around 4, 6 and 10. Below pH 6, exchange is due to carboxyl groups; between 6 and 8.5, to imidazol groups; and, above 8.5, arises from amino groups. Peptides have similar zones of proton exchange.

Sea water is an ideal environment for the natural occurrence of carbamino carboxylic acids. The pH and temperature are nearly optimal and the CO_2 content is high. Organic material from a sample of surface sea

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water has been found by Park and Hood (1959) to have a proton exchange capacity of 0.15 meq/L/ΔpH in the range of 7.8 to 9.0. Whether this material is amine, carboxylic, or a combination of both has not been established. In general, however, organic material isolated from open sea water has been found by Jeffrey and Hood (1960) to contain an organic carbon to nitrogen ratio of about 14 to 1 with a carbon content of 1 to 10 mg/L; thus, a concentration of 0.07 to 0.7 mg of organic nitrogen might be expected.

On the above evidence, carbamino carboxylic acids may be present in sea water, and so, may have biological as well as chemical oceanographic significance. We found (Exp. 1) that addition of the calcium salt of DL-alanine-N-carboxylate to an organic medium reduced the uptake of inorganic carbon by *Nitzschia closterium* (Ehr.) Wm. Smith, *Platymonas* sp. and *Chlorella* (marine) when grown in completely inorganic medium. Since this occurred despite the continuous evolution of oxygen and a uniform increase in total cell count, we were led to consider the possibility of preferential utilization of this complex over inorganic carbon in photosynthesis. The results of experiments to determine the ability of phytoplankton to utilize carbamino carboxylic acids in photosynthesis are presented below.

METHODS

Preparation of Organic Derivatives Used

Carbamino carboxylates were prepared in a 250 ml three-necked flask fitted with a stirrer, an inlet for addition of gaseous CO_2, pH electrodes which were connected to a Beckman Model G pH meter, and a burette for addition of 0.5 N NaOH to maintain the proper pH for the reaction. The temperature was held at 25 + 0.2°C during the preparation.

The alanine derivative (Ferguson and Roughton 1935 and Stadie and O'Brien 1936) was prepared by addition of 0.0022 moles of CO_2 gas to 0.0025 moles DL-alanine in 100 ml of distilled water. The pH was held at 10.5 during the reaction by addition of NaOH which was free of CO_2. $CaCl_2$ (0.0045 moles) was then added to form the calcium salt and precipitate excess $CO_3^=$ ion. The solution was centrifuged (temperature held at 25 ± 0.1°C), and a portion of the supernatant was adjusted to pH 8.2 and added to the experimental culture solutions.

Concentrates of organic materials from natural sea water were prepared in two ways. Concentrate A was prepared from 400 gal of unfiltered surface water collected at a 1000-fathom station in the northern Gulf of Mexico. This preparation was made by coprecipitation of the organic material with ferric and magnesium hydroxide at pH 10; removal of iron by extraction from 3 N HCl solution with isopropyl ether; and, removal of acid and reduction of volume from 10 L to 3 L by vacuum distillation. The concentrate contained excess Ca and Mg over sea water but about the same concentration of NaCl.

Concentrate B was prepared by electrodialysis (Jeffrey and Hood 1960) of 10 gal of sea water to a salt content of 1‰. The volume was reduced by vacuum distillation and the solution was again dialyzed to 1‰. This process was repeated to give a final volume of 580 ml and 1‰ Cl.

The N-carboxylate derivative was prepared by addition of 0.0022 moles of CO_2 to an aliquot of each of the above concentrates. The pH was held at 8.5 in a similar way to that for the alanine derivatives. A portion of this preparation was then adjusted to pH 8.2 and added to the various cultures studied.

The N-C^{14} carboxylate derivatives of alanine and of sea water concentrates were prepared by the exact procedure used for DL-alanine except that the carbon dioxide gas added contained 10 μc of $C^{14}O_2$. A portion of this solution was then added to the cultures studied.

Algal Cultures and Growth Rates

Algal cultures were maintained by weekly subculturing in a medium composed of Ketchum and Redfield's (1938) Sol. A, 2.0 ml; Ketchum and Redfield's (1938) Sol. B, 0.5 ml; Arnon's (1938) micro-nutrients, 0.5 ml; Bold's (1942) soil extract, 2.0 ml; $Na_2SiO_3 \cdot 9H_2O$, 0.0466 gm; $Na_2MoO_4 \cdot 2H_2O$, 4×10^{-5} gm; and, 1000 ml filtered sea

water. Then 24 hr before each experiment, the cultures were centrifuged and washed three times with sterile sea water, and finally subcultured in sterile nutrient solution without the soil extract. Additional nutrients of 1×10^{-4} moles KNO_3 and 3.7×10^{-4} moles KH_2PO_4 were added to the medium to insure rapid growth. The media were adjusted to pH 8.2 and handled aseptically throughout the experiment.

Two liters of culture medium in 4-L Erlenmeyer flasks were placed in a rotary shaker and illuminated with 900 ft-c of light energy from fluorescent tubes. In some cases, one inch of paraffin oil was placed on the surface to eliminate gas exchange. Cultures were maintained at ± 1°C.

Cell counts were made by the following procedure. Ten milliliters of culture were pipetted from the shaking flasks at the same time as samples were taken for C^{14} uptake measurements. These cultures were mixed in an omni-mixer for 45 sec to disperse the clumps and a portion was then counted in a hemocytometer.

Five types of experiments were undertaken. In Experiments 1 through 4, growth in the culture was measured by the conventional C^{14} uptake method of Steeman Nielsen (1952). After incubation in a medium to which $HC^{14}O_3$ had been added, subsamples of cultures 25 or 50 ml were withdrawn from the culture flasks, filtered through an HA type millipore filter (Millipore Filter Corp.), washed thoroughly with 1N HCl to remove any adsorbed CO_2, dried at 80°C, and counted in a Nuclear Chicago Model 182A gas flow proportional counter which was found to have a 33% efficiency for C^{14}.

In Exp. 5, both $C^{14}O_2$ assimilation and O_2 evolution were measured. An Aminco Dual-Shaker refrigerated type Warburg constant volume respirometer was fitted with fluorescent lighting tubes and used to facilitate study of some aspects of the problem. Biological oxygen demand flasks of 125 ml capacity, which contained the cultures, were connected to the manometers and placed in the bath held at a temperature of 25 ± 0.05°C. A light energy of 500 ft-c was incident to the bottom of the flasks throughout these experiments. Culturing conditions for the phytoplankton were the same as in previous studies. In this case, 50 ml aliquots of the culture were pipetted into each of the sterile flasks, and 1 ml of a solution of 0.005 M KNO_3 and 0.018 M KH_2PO_4 was added. To estimate C^{14} uptake, the entire flask content was treated and counted as above. Oxygen evolution was determined by the method described by Umbreit, *et al.* (1957).

RESULTS AND DISCUSSION

Experiments with N-Carboxylates of DL-Alanine and Sea Water Concentrates

Exp. 1. The first series of experiments were designed to show the effect of DL-alanine-N-carboxylate on the uptake of inorganic carbon during photosynthesis of *Nitzschia closterium* (Ehr.) Wm. Smith. Two 2-L cultures were labeled with 20μc of $HC^{14}O_3$ and the uptake of C^{14} was determined for a period of time to ascertain that the cultures were healthy and that growth was nearly linear with time. To one of the flasks was then added 10 ml of the DL-alanine-N-carboxylate, whereas, the other was left as a control. Cell counts were also made at various time intervals throughout the experiment. The results of a typical experiment are shown in Figure 1. The addition of DL-alanine-N-carboxylate inhibited the uptake of C^{14} from the labeled inorganic system for a rather extended period (approximately 2.5 hr in case shown) after which uptake returned to approximately the same rate as before. Cell counts indicated a continuous linear increase in cell numbers through the experiment. Similar results were obtained with *Platymonas* sp. and *Chlorella* (marine).

Exp. 2. The purpose of this experiment was to investigate the N-carboxylate type compounds of sea water as inhibiting agents to utilization of inorganic forms of carbon by *N. closterium* in photosynthesis.

This experiment differed from the first in that the culture medium was covered with a layer of paraffin oil to allow measurements of oxygen as an index of continuous growth. Alanine, CO_2 and Ca were added

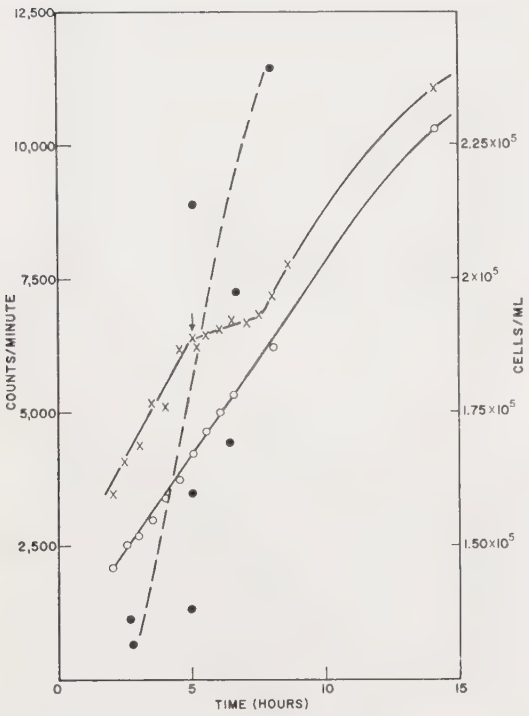

FIG. 1. Effect of N-carboxylates of DL-alanine on C^{14} uptake by *N. closterium*. • — • = cell counts/ml; o — o = control; x — x = DL-alanine-N-carboxylate.

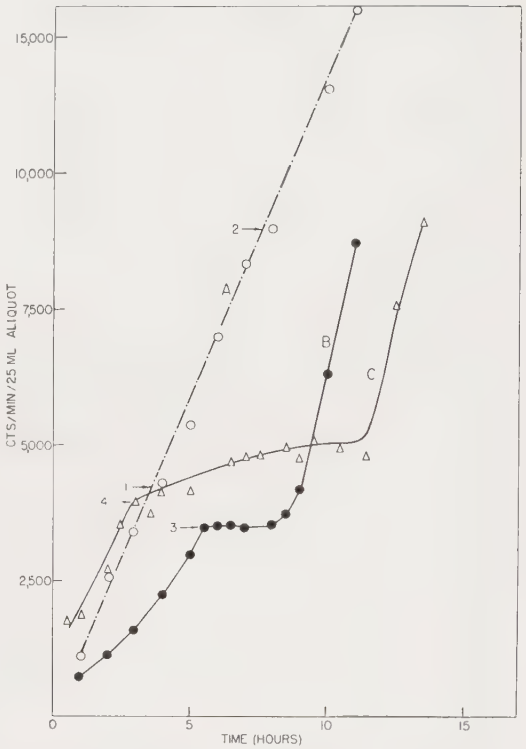

FIG. 2. Effect of N-carboxylates of sea water on C^{14} uptake by *N. closterium*. o — o = control; Δ — Δ = N-carboxylates of sea water; • — • = N-carboxylates of alanine.

to the control in place of DL-alanine-N-carboxylate. The results are shown in Figure 2.

The addition of DL-alanine-N-carboxylate inhibited the uptake of inorganic C^{14} for about 3 hr, while the addition of N-carboxylate concentrated from 10 gal of sea water (Concentrate B above) caused the inhibition of inorganic C^{14} uptake for about 8 hr. Subsequently, growth was resumed at its previous rate in both cases. The oxygen concentration in all the cultures increased at a steady rate and did not reflect the periods of inhibition of assimilation of inorganic carbon.

It would appear, therefore, that the N-carboxylate derivatives of both DL-alanine and of organic concentrates from sea water are used in preference to inorganic carbon in photosynthesis by certain marine phytoplankton. Growth appeared to continue during the inhibition period at a constant rate as indicated by both oxygen evolution and cell counts. Neither alanine alone nor CO_2 and Ca were found effective in producing this inhibition.

Exp. 3. To investigate which portion of the N-carboxylate molecule was utilized in photosynthesis, the third experiment was undertaken. The culture conditions were the same as in the first experiment except that inorganic C^{14} was added to only the control culture. To a second flask was then added 50 ml of a preparation of DL-alanine-N-C^{14} carboxylate which contained 0.16 μc activity, and the rate of C^{14} uptake was determined as before.

It is seen from Figure 3 that uptake of C^{14} from the organic source occurs immediately after addition of DL-alanine-N-C^{14} carboxylate and continues in an almost linear fashion for a period of 15 hr. In a similar situation when only 5 ml of the labeled derivative was added, the uptake leveled off approximately 5 hr after addition.

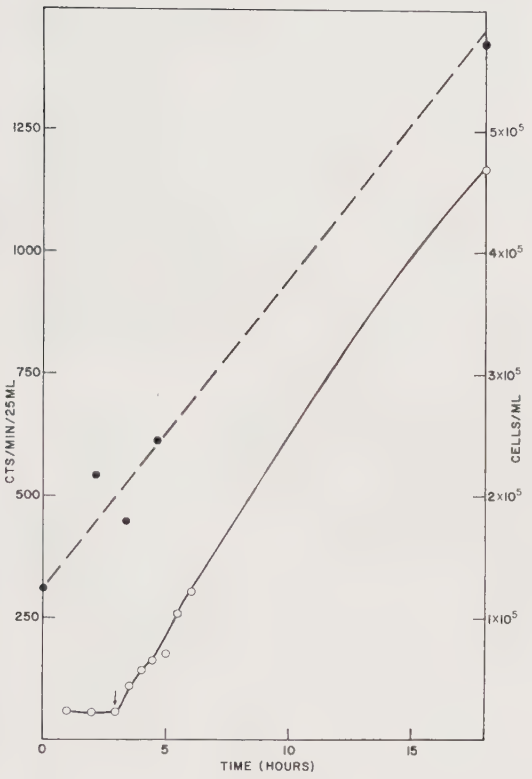

FIG. 3. Uptake of C^{14} from DL-alanine-N-C^{14} carboxylate. • — • = cell counts/ml; o — o = C^{14} uptake by *N. closterium*.

The total activity taken up by the algae during the growing period was 0.13 μc of C^{14} or approximately 80% of the total activity added. In the control, only 6% of the inorganic activity was taken up in the same period.

The results of this experiment clearly indicate that the N-carboxylate moity of the DL-alanine-N-carboxylate is utilized in photosynthesis by the marine algae tested.

Exp. 4. The possibility yet remains that the alanine moity may also be used as a source of carbon when associated with the carboxylate derivative. This experiment was identical to Exp. 3 except that DL-alanine-1-C^{14} containing 6 μc of C^{14} was used in the preparation of the N-carboxylate derivative. The results of this experiment are given in Table 1.

It appears that the carboxyl group of DL-alanine does not replace inorganic carbon

TABLE 1. *Uptake of DL-alanine-1-C^{14} N carboxylate by* N. closterium *under light and dark conditions*

Hours	Incident Light (ft-c)	DPM
0	900	210
1	900	2496
3	900	2451
6	900	2433
6 to 20	0	4908
20 to 23	900	4833

for photosynthesis in *N. closterium*. The rapid initial uptake is probably due to adsorption on the algae. This phenomenon would not be evident in the third experiment since any surface adsorbed N-C^{14} carboxylate would be decomposed by the wash conditions used in preparation of the sample for counting.

The uptake of the DL-alanine-C^{14}-N-carboxylate in the dark is of interest, but is a topic for consideration in another study. DL-alanine-1-C^{14} alone added to cultures showed almost identical results to those in Table 1.

Utilization of Other Organic Compounds

To determine the effect of other compounds in the transfer of CO_2, experiments using the Warburg apparatus in place of the large culture flasks were conducted. This apparatus allowed measurement of oxygen evolution throughout the experiment as an index of growth, and also provided constant temperature and shaking conditions for 14 simultaneous samples.

Exp. 5. In this experiment, 50 ml of a culture of *N. closterium* containing 2.5×10^7 cells was added to each flask together with 0.1 μc of C^{14} and 2.5×10^{-5} moles of the test compounds. Oxygen evolution was measured at 30-min intervals throughout the experiment. After a period of one hour the total contents of the flasks were filtered, and the amount of C^{14} assimilated was determined as before. The results are given in Table 2.

Of those compounds tested, only DL-alanine-N-carboxylate and ammonium bicarbonate reduced the uptake of inorganic

TABLE 2. *Effect of other organic compounds in replacing inorganic carbon for photosynthesis. Oxygen evolution was between 30 and 40 μl/hr for all cultures*

	pH	CPM*
Carbamyl-phosphate	8.15	447
Carbamyl-DL-aspartate	8.1	570
Carbamyl-L-glutamate	7.9	440
Citrate	8.0	510
Ammonium bicarbonate	7.9	147
Ammonium chloride	8.0	360
DL-alanine-N-carboxylate	8.0	206
DL-alanine + calcium chloride	8.1	478
Control	8.1	500

* average of duplicate samples.

carbon from the medium. The effect of the latter compound is probably due to its equilibrium product, ammonium carbamate, which is always present in a commercial preparation of the ordinary carbonate (Ephraim 1939).

Only a few compounds were tested in this preliminary study. Of primary interest were the carbamyl derivatives of glutamic and aspartic acid, and the carbamyl phosphate which differ in structure from the N-carboxylate derivatives by having an amide nitrogen on the N-carboxylic group. Carbamyl-L-glutamic acid has been shown by Cohen and Grisolia (1948, 1950) to be functional in CO_2 transfer in the synthesis of arginine from ornithine by rat liver tissue but was found by Hood and Lyman (1950) to be nonfunctional in the same synthesis by *Lactobacillus arabinosus.*

Further tests of this type may reveal other compounds capable of replacing the N-carboxylate derivatives, but it would already appear that the algae have a high degree of specificity for the N-carboxylate group in photosynthesis.

DISCUSSION

The total CO_2 content of sea water at a pH of 8.2 and a chlorinity of 19‰ is about 2.4×10^{-3} moles/L. It seems reasonable to expect that at least 1 to 3×10^{-6} moles/L of complexed CO_2 may exist under open sea conditions and considerably greater amounts would be expected in the nearshore environments. In the open sea this represents about 10 to 30% of the concentration of molecular CO_2 or about 0.1% of the CO_2 present in all forms. The quantitative evaluation of the complexed CO_2 present in various marine environments will be the subject of a later paper.

Phytoplankton may utilize complexed CO_2 in preference to inorganic forms since it serves as a more ready source of CO_2 being adsorbed to the cell wall, or because it is more permeable to the cell membrane. Our evidence suggests that the mechanism of utilization of CO_2 from the inorganic system involves a complexing with proteinaceous material. The complex when adsorbed on the cell wall may readily give up CO_2 by addition of hydrogen ion and the release of the complexing agent. Such a mechanism would then parallel a similar system which has been shown to occur in higher animals (White, *et al.* 1959) during transport of CO_2.

In primary production measurements by the C^{14} uptake method described by Steeman Nielsen (1952) the complexed CO_2 may play a role in associating respired CO_2 from the photosynthesizing plants and by its preferential uptake over inorganic CO_2 give the "net" production values observed. This fact alone would cause no errors in the measurements but, should bacteria and animals present also produce a respired complex which in turn would be used by the plants preferentially, some error would be expected. Further elucidation of the chemistry involved will be necessary in order to evaluate this point.

It is possible that complexed CO_2 may be involved in many geochemical or physical processes that involve CO_2 deposition, utilization, or mass transfer in the environment. It is therefore significant that work is continuing on determination of the amounts present and on studies of the mechanism and kinetics of its reactions.

REFERENCES

ARNON, D. I. 1938. Microelements in culture solution experiments with higher plants. Am. J. Bot., **25**: 332.

BOLD, H. C. 1942. The cultivation of algae. Bot. Rev., **8**: 66–138.

COHEN, P. P. AND S. GRISOLIA. 1948. Intermediate role of carbamyl-L-glutamic acid in citrulline synthesis. J. Biol. Chem., **174:** 389–390.

———, ———. 1950. The role of carbamyl-L-glutamic acid in the enzymatic synthesis of citrulline from ornithine. J. Biol. Chem., **182:** 747–761.

EPHRAIM, FRITZ. 1939. Inorganic Chemistry. Nordman Publishing Company, Inc., New York, 3rd ed., 867 pp.

FAURHOLT, C. 1924. Etudes sur les solutions aqueuses de carbamates et de carbonates. J. Chim. Phys., **21:** 1–44.

FERGUSON, J. K. W. AND F. J. W. ROUGHTON. 1935. The direct chemical estimation of carbamino compounds of CO_2 with haemoglobin. J. Physiol., **83–84**, 68–102.

HOOD, DONALD W. AND CARL M. LYMAN. 1950. The role of glutamic acid in arginine synthesis by *Lactobacillus arabinosus*. J. Biol. Chem., **185:** 39–44.

JEFFREY, LELA M. AND DONALD W. HOOD. 1960. Organic matter in sea water; an evaluation of various methods for isolation. J. Mar. Res. (In Press.)

JENSEN, A. AND C. FAURHOLT. 1952. Studies on Carbamates. V. The carbamates of α alanine and β alanine. Acta. Chem. Scand., **6:** 385–394.

KETCHUM, B. H. AND A. C. REDFIELD. 1938. A method of maintaining a continuous supply of marine diatoms by culture. Biol. Bull., **75:** 165.

NEUBERG, CONRAD, A. GRAUER, AND M. KREIDL. 1955. The basis of the carbamate reaction. Arch. Biochem. Biophys., **58:** 169–177.

———, ———, ———, AND H. LOWRY. 1957. The role of the carbamate reaction in the calcium and phosphorus cycles in nature. Arch. Biochem. Biophys., **70:** 70–79.

PARK, KILHO AND DONALD W. HOOD. 1959. Effect of organic material on the solubility of calcium carbonate in sea water. Preprints of the International Oceanographic Congress, New York. (1959) p. 873.

SNEDECOR, G. W. 1946. Statistical Methods. Iowa State College Press, Ames, Iowa.

STEEMANN–NIELSEN, E. 1952. The use of radioactive carbon (C14) for measuring organic production in the sea. J. Cons. Internat. Explor. Mer., **43:** 117–140.

STADIE, W. C. AND HELEN O'BRIEN. 1936. The carbamate equilibrium. I. The equilibrium of amino acids, carbon dioxide and carbamates in aqueous solution with a note on the Ferguson–Roughton carbamate method. J. Biol. Chem., **112:** 723–758.

UMBREIT, W. W., R. H. BURRIS AND J. F. STAUFFER. Manometric Techniques. Rev. ed. Burgess Publishing Co., Minneapolis, Minn., 1957.

WHITE, ABRAHAM, PHILIP HANDLER, E. L. SMITH AND DEWITT STETTER, JR. 1959. Principles of Biochemistry, McGraw-Hill Book Company, Inc., New York, 1149 pp.

NOTES AND COMMENT

Caulobacter sp. In Sea Water

Jannasch and Jones (1959) reported finding "spirilli-like" bacterial forms in sea water using a direct microscopic method. At one station the "spirilli-like" organisms comprised from 13 to 45% of the open ocean bacterial population as determined by the Cholodny method. Their abundance was inversely proportional to the remaining bacterial population. We now present evidence to identify these organisms as members of the genus *Caulobacter*. The only previous indication that others may have observed *Caulobacter* in the sea was the statement by ZoBell and Upham (1944 p. 253) that stalked bacteria exist in sea water.

Caulobacter is a common freshwater microorganism, having been observed in nonpolluted waters by various investigators using the submerged slide technique. The identification of Caulobacteraceae is possible by a morphological feature of remarkable constancy, the stalk formation. On this basis, Henrici and Johnson (1935) established the taxonomy of the group without pure cultures or extensive cultivation on differential media. Their concept has been accepted and is still valid (Bergey 1957). In the earliest observations of these microorganisms, Jones (1905) was able to observe the stalks, and, after common staining, decided they were true flagella. From microscopic examination of submerged slides, Henrici and Johnson (1935) concluded that "ferric hydroxide or other material is secreted from one side or one end of the cell to form the stalk." Houwink (1955), however, after detailed examination under the electron microscope, suggested that the stalk was part of the living cell.

Electron photomicrographs (Fig. 1) were prepared from concentrated and desalted samples of sea water, using a protein monolayer technique (Hartman *et al.* 1953). The morphological features of the "spirilli-like" organisms clearly resembled the electron photomicrographs of *Caulobacter* presented by Houwink (1955). Stalks of different lengths in our electron photomicrographs

Fig. 1. Electron micrograph of *Caulobacter sp.* concentrated by the cholodny technique from sea water taken 16 miles off San Diego at a depth of 75 m. Magnification × 9000.

suggested fragmentation of the cells, possibly during preparation. "Button-like" holdfasts such as those reported associated with *Caulobacter* in fresh water were not observed in our preparation. We observed nothing to indicate that *Caulobacter* was parasitized in the open ocean; it was numerous, and other microorganisms were not attached as observed in the electron photomicrographs. We believe that the morphology of the "spirilli-like" microorganisms in our preparations is sufficient to classify them as *Caulobacter*. Additional work is needed on the physiology, geographical distribution, and ecology of these prominent members of the marine environment.

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REFERENCES

BERGEY'S MANUAL OF DETERMINATIVE BACTERIOLOGY (1957). 7th ed. Edited by Breed, Murray, and Smith. Williams and Wilkins Co., Baltimore, Md., pp. 212–214.

HARTMAN, R. E., T. D. GREEN, J. B. BATEMAN, C. A. SENSENEY, AND G. E. HESS. 1953. Preparation of uniformly dispersed specimens of particulate matter for electron microscopy. J. Appld. Physics, **24**: 90–92.

HENRICI, A. T. AND D. E. JOHNSON. 1935. Studies of freshwater bacteria II. Stalked bacteria, a new order of Schizomycetes. J. Bacteriol., **30**: 61–94.

HOUWINK, A. L. 1955. *Caulobacter*, its morphogenesis, taxonomy and parasitism. Ant. van Leeuwenhoek, **21**: 49–64.

JANNASCH, H. W. AND G. E. JONES. 1959. Bacterial populations in sea water as determined by different methods of enumeration. Limnol. Oceanogr., **4**: 128–139.

JONES, M. 1905. A peculiar microorganism showing rosette formation. Centralbl. f. Bakt. II, **14**: 459–463.

ZOBELL, C. E. AND H. C. UPHAM. 1944. A list of marine bacteria including descriptions of sixty new species. Bull. Scripps Inst. Oceanogr., **5**: 239–292.

AMERICAN INSTITUTE OF BIOLOGICAL SCIENCES TRANSLATION PROGRAM

The American Institute of Biological Sciences is currently translating and publishing seven Russion research journals in biology. These journals are translated with support from the National Science Foundation, which is eager that such information be more widely distributed to biologists throughout the world. It is hoped that this material will aid biologists in research, prevent duplication of work, give some idea of the work being done by Soviet scientists in the field of biology, and also bring about a better international understanding among scientists.

Because of the support of the National Science Foundation, the AIBS can offer these translations at a fraction of their publication cost, with even further price reduction to AIBS members and to academic and nonprofit libraries. This reduction, the AIBS feels, places the translation within the reach of all biologists.

The journals currently being translated are: Doklady: Biological Sciences Section; Doklady: Botanical Sciences Section; Doklady: Biochemistry Section; Plant Physiology; Microbiology; Soviet Soil Science; and Entomological Review.

In addition to its program of Russian Biological Journal translations, the AIBS has instituted a separate program of translation and publication of selected Russian Monographs in biology.

It was felt that the program of Journal translations was not sufficient to cover all of the significant work being done in all fields of biology by Russian scientists. With the aid of competent authorities, the AIBS has translated and published six Russian monographs and one monograph is in the process of being published. In addition, several prominent monographs in various biological areas are being considered by the AIBS and the National Science Foundation for translation and publication. The monographs that have been published are: Origins of Angiospermous Plants by A. L. Takhtajan; Problems in the Classification of Antagonists of Actinomycetes by G. F. Gauze; Marine Biology, Trudi Institute of Oceanology, Vol. XX, edited by B. N. Nikitin; Arachnoidea by A. A. Zakhvatkin; and Arachnida by B. I. Pomerantzev. The manuscript for Plants and X-rays by L. P. Breslavets is in the final stages of preparation and should be published early in 1960.

Additional information pertaining to this program may be obtained by writing to the American Institute of Biological Sciences, 2000 P Street, N.W., Washington 6, D. C., U. S. A.

Botanica Marina

"Botanica marina" is a new international journal devoted to the taxonomy, morphology, ecology, physiology and chemistry of seaweeds and other marine algae. Each yearly volume will have four issues of 32 pages: additional issues will be devoted to the industry and technology of seaweed products. The first two issues include the following interesting papers:

Biebl, R., Zellphysiologische Beobachtungen an Blasenzellen einiger Rotalgen

Soderstrom, J., A Swedish find of *Antithamnion cruciatum* (Ag) Naegeli ∝ *genuina* Kolderup Rosenvinge

Bergquist, P. L., A statistical approach to the ecology of *Hormosira banksii*

Schmid, O. J., Marine Rotalgen. Chemische Zusammensetzung

Manuscripts should be sent to: Professor T. Levring, Marinbotaniska Institutionen, Botaniska Tradgarden, Göteborg, Sweden. The yearly subscription of DM.28 should be sent to Verlag Cram, De Gruyter & Company, Hamburg 1, West Germany.

Symposium on Marine Microbiology

Sixty microbiologists—one half from foreign nations—will present reports of their research at a Symposium on Marine Microbiology in the Morrison Hotel, Chicago, April 20–24, 1961.

The symposium is being sponsored by the Office of Naval Research and the Society of American Bacteriologists in cooperation with the American Institute of Biological Sciences. Program chairman is Dr. Carl H. Oppenheimer of the University of Texas Institute of Marine Science, Port Aransas, Texas.

Technical papers will be presented on autotrophic and heterotrophic micro-organisms, nutritional symbiosis between marine organisms, mineralization activity, fungi, viruses, algae, protozoa and phytoplankton and their ecological relationships. Basic research in these areas is expected to provide information which may lead to more complete utilization and controlled production of food materials, antibiotics, pharmaceuticals, and other important products of the oceans resulting from microbial activity.

Dr. Jacques Senez of Marseille, France, a marine microbiologist noted for his expositions on the philosophy of science, will deliver the annual ONR lecture as part of both the symposium program and the S.A.B. meeting. Dr. Senez also will serve as a section chairman for the symposium program.

Other section chairmen include: Prof. Wilhelm Schwartz, University of Greifswald, Greifswald, Germany; Dr. E. J. Ferguson Wood, Commonwealth Scientific and Industrial Research Organization (CSIRO), Cronulla, Australia; Dr. Ronald Hayes, Dalhousie University, Halifax, Nova Scotia; Dr. Luigi Provasoli, Haskins Laboratory, New York, N.Y.; Dr. S. C. Rittenberg, University of Southern California, Los Angeles; Dr. W. D. McElroy, Johns Hopkins University, Baltimore, Md.; and Dr. C. E. ZoBell, Scripps Institution of Oceanography, LaJolla, Calif.

BOOK REVIEWS

EDMONDSON, W. T., editor. 1959. **Fresh-Water Biology.** 2nd edition. John Wiley: New York. xx + 1248 pp. Price $34.50.

The first edition of "Fresh-Water Biology" (1918) was a pioneer attempt to bring together in one book keys for the identification of the organisms occurring in the inland waters of North America north of Mexico. Being not too expensive, it was readily available to the average person interested in aquatic natural history and systematics, and as a result it was used extensively by many generations of college and even high school students. Moreover, it also has enjoyed a wide usage among professional biologists as a convenient source of reference. Its impact in the entire field of aquatic studies has been very great.

In the subsequent four decades great advances have been made in our knowledge of the systematics of the various groups and the distribution of the individual species. Understandably, as a result there has been agitation for a long time for a revision of this very useful work, which has at last been accomplished under the capable direction of W. T. Edmondson.

The desire to incorporate all this new information into a single volume has presented many problems, chief of them being limitation of space. Pagewise the revision is only 12% larger than the original edition. However, a larger page gives an increase of 45% in total information that can be presented with the same typography, and even more than that by using various devices of compaction and selection of type. Even so, the approximately 50% increase in size of the book is inadequate for all the new information accumulated, and something has had to be sacrificed. Certain chapters in the original edition—two general chapters, the treatments of the parasitic flatworms and roundworms, and the chapter on aquatic vertebrates—have been eliminated. Moreover, the introductory material in each chapter has been reduced to a minimum, so that the revision even more so than the original edition is essentially an aid to identification of organisms.

Of the 46 chapters in the new edition, 42 present keys to particular groups of organisms at the phylum, class, subclass, or order level, depending on the size of the taxon involved. The other four consist of introductory chapters on the Protista and insects, a general introduction to the book, and a concluding chapter on methods and equipment. Each of the chapters has been assembled by one or more specialists in the pertinent group of organisms, and hence can be presumed to be authoritatively presented. They are well done and will be very useful. There are valuable new chapters on the aquatic fungi, polychaetes, tardigrades, and bryophytes, and expanded systematic treatments of the vascular plants, bacteria, and aquatic insects. Within the space limitations of this volume a veritable mountain of information has been presented to aid in the identification of freshwater organisms.

The treatment of the various groups of organisms to genus or species is in general the same in the two editions, although the bluegreen algae which were keyed only to genus in the first edition are keyed to species in the revision. Each specialist was apparently given full responsibility as to how he would handle the group within the number of pages allotted him.

In retrospect one wishes that Edmondson had attempted to obtain a little more uniform treatment of the various taxa. The minimum treatment for each group is a key to the genera, with an illustration and description of a typical species in each. Some chapters, such as those on the rhizopods, ciliates, rotifers, and Nemata, give in addition the number of species in each genus currently known from North America. In other chapters, such as those on aquatic fungi, zooflagellates, diatoms, and other algae excluding bluegreens, bryophytes, etc., this helpful information is not given. Persons using any of these generic treatments will have to consult more detailed treatises or enlist the aid of specialists in identifying particular species. In any case, as Edmondson points out in the introduction, the keys should serve only as guides, and any critical identifications should be substantiated by more detailed study or done by specialists.

The best over-all section in the book is that on the Crustacea. All treatments are to species, except the parasitic copepods, amphipods, isopods, and Pacific Coast crayfishes, which are taken only to genus. This is regrettable, since with only a small amount of expansion one would have a complete treatment of the non-parasitic Crustacea, comparable to some of the monographic works published in Europe [*e.g.* Wagler's "Crustacea (Krebstiere)," in Die Tierwelt Mitteleuropas].

The chapter on aquatic insects in the original edition was formidable to the average person because of the almost complete lack of illustrations. In the revision each major order is treated separately by a specialist, with good illustrated keys to the aquatic stages. Space limitations have prevented consideration of the adults, except among the Hemiptera and Coleoptera where the adults as well as the immature stages occur in water. Persons interested in a comprehensive generic treatment of the adult terrestrial stages will probably wish to use "Aquatic Insects of California" by Usinger *et al.*

Smaller groups of organisms, including the Turbellaria (except for subterranean Kenkiidae) and the oligochaetes (except for some of the more complex forms), are keyed to species. These treatments will be very useful to workers in the groups.

One of the chief deficiencies of the revised edition is the quality of the illustrations. Wherever possible the same figures used in the original edition have been employed in the revision. These and

other figures borrowed from the literature suffer in loss of the detail that is essential in taxonomic literature. On the other hand, the new illustrations in the chapters on aquatic fungi, algae, and zooflagellates, for example, are excellent.

An even more serious drawback is the price, which is beyond the means of the ordinary person who might otherwise have occasion to use the book. This volume is intended to reach a wide audience of amateurs and professionals, as was the original edition, and does not presume to take the place of monographic faunistic and floristic treatises. However, some of the chapters, such as those on the various groups of the Crustacea, are almost of this scope in that they present keys not available anywhere else in the literature to all the species known from North America at present. A person interested specifically in these chapters, or in those on the rotifers, aquatic mites, etc., is penalized financially for all the other keys that are available elsewhere in more exhaustive treatments of the various groups.

To help increase the use of this material by the wide audience intended, one possible solution might be the release of individual chapters or groups of related chapters in paperback form. Certainly the treatments of the Aquatic fungi, Algae, Protozoa, Turbellaria, Gastrotricha and Rotifera, and Crustacea might well enjoy a considerable market demand in this form.

Another problem concerns the future. Systematics is a dynamic field, continually changing as more information accumulates and as new techniques are developed. Will there eventually be a third edition of this book? To the reviewer this seems unlikely. What we need henceforward is a more flexible and responsive system for the printing and distribution of revised treatments of the individual groups as the need for their revision becomes apparent.

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